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TYPES OF VEGETATION IN THE SEMIARID PORTION OF THE UNITED STATES AND THEIR ECONOMIC SIGNIFICANCE[1]

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The natural or normal plant cover is a result of all growing conditions of the area where it is produced. It is therefore an index or measure of the factors influencing its growth and it serves as an indicator of the possibilities of producing other plants on the land. The differences in growing conditions have resulted in various plants and groups of plants. A number of these have been recognized as having indicator value in determining the use that can be made of the land for growing farm crops. Some plants can thrive in a wide range of growing conditions; others, however, are affected by comparatively small changes. In the various plant communities or types some species may drop out and others come in as a result of changes in growing conditions. Better or less favorable growing conditions in a region are also indicated by differences in the density of the vegetation within the same type.

In the following paper the principal types of vegetation occurring on the unreserved public lands and the patented homestead lands west of the one hundredth meridian are briefly discussed with special reference to their economic significance to crop production and grazing and some of the more important ones are illustrated. These types have been recognized and used in connection with data on topography, soil, climatic conditions, accessibility, and past agricultural history to determine the value of the lands for the production of cultivated crops and for grazing. At the time the land classification work was started there were numerous areas that had not been settled or where settlement had been so recent that no agricultural history was available. In most of these areas definite climatic data were also lacking. The vegetation therefore served as the principal means of determining the relative value of these lands for farming.

The expression "vegetation type" is here used in the sense employed by foresters to indicate a plant community of any size, rank, or stage of development. It is synonymous with the technical phytogeographic term "plant community."

In the consideration of the suitability of land for grain farming, forage, or grazing, the following 102 vegetation types have been used, which it is believed represent the most important ones to be found in the region covered by the paper. The key (pp. 117–119) summarizes the types under headings indicating their value for the production of grain or cultivated forage crops or for grazing. Owing to the fact that all the types of vegetation are used for grazing,

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an additional key has been prepared arranging the types according to their approximate carrying capacity. This was based on the average period of the time each year that the vegetation can be grazed without injury.

To aid in the use of the vegetation types, especially to show their range, a map was prepared dividing the western part of the United States into 15 regions, each having in a general way a similarity in vegetation. A copy of this map, together with a list of the types giving their distribution in the various regions, follows the text.

(1) GRAMA GRASS (*Bouteloua gracilis* (H. B. K.) Lag.).—The grama grass type shown by Plate 1, A, is probably the most common and abundant plant type throughout the northern and western Great Plains region. It also extends westward into the semidesert areas on the high plateaus of New Mexico, Arizona, southern Colorado, and southeastern Utah. It is composed of a relatively even stand of grama grass, presenting to the eye no other prominent plants, and grows in a rather compact fertile loam soil usually known locally as hard or tight land. The rainfall over the region occupied by this type is usually between 10 and 15 inches which penetrates the soil to a depth of 1 to 1½ feet. Areas characterized by the grama grass type of vegetation will produce good crops of wheat and other small grains only during years of more than normal rainfall. As grazing land this type is good, having a carrying capacity of from 20 to 40 head of cattle per section.

(2) GRAMA GRASS AND WHEATGRASS (*Agropyron smithii* Rydb.).—The rather heavy clay loam soils in the northern Great Plains are mainly vegetated by the grama grass and wheatgrass type, which is composed of about equal amounts of grama grass and wheatgrass. The heavier the texture of the soil, the greater the amount of wheat grass present in the type, while on the lighter soils grama grass is dominant. The heavy soil makes crop production doubtful in dry years, but relatively heavy in wet years. Considerable native hay is cut from this type. It also provides a large amount of excellent grazing, especially for cattle and horses. The number of stock that it will graze varies considerably according to the density of the vegetation but most of the lands will carry from 30 to 60 head of cattle per section.

(3) GRAMA GRASS AND NIGGERWOOL (*Carex filifolia* Nutt.).—A more or less equally mixed plant cover of grama grass and niggerwool is a very common type in the northern Great Plains. The latter species is characterized by innumerable black roots which form a dense tough sod. This type grows in well-drained loam soils, frequently where growing conditions are less favorable than for the preceding type owing to shallow or rocky soils, or to a lower precipitation. Crops can only be produced on lands supporting this vegetation during exceptionally good years. Niggerwool has a very short growing season and produces its growth early in the spring. This type is not so good grazing as Type (1), but will usually carry from 20 to 40 head of cattle per section.

(4) GRAMA GRASS, NIGGERWOOL, AND JUNEGRASS (*Koeleria cristata* (L.) Pers.).—An even mixture of grama grass and niggerwool containing an open stand of Junegrass is a common type in well-drained fertile loam soils of the northern Great Plains. A large acreage of this type has been placed under cultivation with fair success in all but the drier years. It also provides fair grazing, having a carrying capacity ranging from 30 to 60 head of cattle per square mile during the average year.

(5) GRAMA GRASS AND MOUNTAIN SAGE, also known as FRINGED WORMWOOD (*Artemisia frigida* Willd.).—This type, illustrated by Plate 1, B, is composed of a relatively pure stand of grama grass with scattered overgrowth of mountain sage. It is found in rather limited areas in the northern Great Plains occupying the higher grasslands, mesas, mountains, and valleys, as far south

as New Mexico, usually occupying smooth bench lands having a rather coarse loam soil. It is also characteristic of areas of land once plowed and revegetated, and of overgrazed areas. It characterizes land where the moisture supply is in excess of the requirements of the grama grass. This may be due to unusually heavy rainfall or to the lessened demand of the grass due to overgrazing. The latter causes the mountain sage to increase at the expense of the grama grass. As agricultural land it will produce good crops of cool-weather cereals, such as wheat and oats, except during years of less than normal rainfall. The carrying capacity of this type ranges from 25 to 50 head of cattle per section.

(6) GRAMA GRASS, NIGGERWOOL, AND SAGEBRUSH (*Artemisia tridentata* Nutt.).—Plate 2, A, illustrates this type which consists of an open stand of sagebrush growing over a comparatively even stand of grama grass and niggerwool. Frequently this type will appear as alternating small areas of relatively pure short grass and sagebrush. Common on friable loam soils in central and south-central Wyoming and northwestern Colorado. It occurs chiefly in regions where the rainfall is intermediate in character between the spring and summer type of the Great Plains and the year-round type of the Great Basin. Agriculture is very doubtful. An occasional crop may be grown during unusually good years. As grazing land it supplies considerable feed but has a low carrying capacity, ranging from 15 to 25 head of cattle per section.

(7) GRAMA GRASS AND SAGEBRUSH.—This type is usually made up of a rather thin stand of grama grass over which are growing varying densities of sagebrush ranging from a thick to a scattering stand. It also contains alternating pure patches of sagebrush and grama grass. It is very common in southwestern Colorado, southeastern Utah, and on the higher plateaus in northwestern New Mexico, in regions where the mean annual precipitation ranges from 14 to 18 inches. It may also contain varying amounts of cedar and piñon, either scattered in clumps throughout the type or bordering it on the rough lands. This type covers a rather wide range of agricultural possibilities. If, however, the sagebrush makes a thrifty rank growth the type would indicate very favorable possibilities for agriculture, including the production of small grains. The carrying capacity varies considerably, depending largely upon the amount of grama grass that is present, the sagebrush having little forage value except during the late fall and winter for sheep. The average area will graze 20 to 30 head of cattle per section.

(8) GRAMA GRASS AND CHAMISO[2] (*Atriplex canescens* (Pursh) James).—The grama grass and chamiso type grows in a clay soil and is widely distributed throughout the western Great Plains and plateau lands of Colorado, Arizona, New Mexico, and Utah. It is composed of a comparatively open stand of grama grass containing chamiso scattered over it at intervals of 10 to 100 feet. As grazing land it will carry from 15 to 30 head of cattle per section, but it is very doubtful if it will produce much additional feed when placed under cultivation.

(9) GRAMA GRASS AND WILD ALFALFA (*Psoralea tenuiflora* Pursh).—Plants of "wild alfalfa" (also known as scurf-pea) in this type are scattered over a sod composed principally of grama grass. It indicates a relatively abundant water supply in the deeper soil, either due to precipitation or flood water. Common on black loam soils in the northern and western Great Plains area. As agricultural land it is reasonably sure to produce a crop of wheat during years of average or more than average moisture supply. The carrying capacity of lands producing grama grass and wild alfalfa varies from 50 to 90 head of cattle per section.

(10) GRAMA GRASS AND MATCHWEED (*Gutierrezia sarothrae* (Pursh) Britten & Rusby).—Matchweed, a low, yellow-flowered shrub, 3 to 12 inches high, is scat-

[2] This is also known as fourwing saltbush.

tered more or less equally over grama grass in this type. Matchweed is especially abundant in rocky or shallow soils. It also comes in on the better lands when the natural vegetation is weakened by overgrazing, or continued drought, or is destroyed by plowing. This type is rather common throughout the central and southern Great Plains. The carrying capacity is low, from 5 to 15 head of cattle per section, and the land is of doubtful agricultural value.

(11) GRAMA GRASS AND VALLEY SAGE (*Artemisia nana* Nutt.).—This type, illustrated by Plate 2, B, is limited largely to the plains region of Montana, growing in a rich deep loam soil in swales and in small valleys. It consists of a scattered growth of valley sage over a grass sod composed mainly of grama grass. Agriculturally, the land will produce during years of more than average moisture supply. The grazing capacity is good, ranging from 30 to 60 head of cattle per section, the number being dependent upon the density of the vegetation.

(12) GRAMA GRASS AND WESTERN NEEDLEGRASS (*Stipa comata* Trin. & Rupr.).—A mixed stand of grama grass and needlegrass, with niggerwool, wheatgrass, and mountain sage, comprise this type. During dry seasons it appears to be composed mainly of grama grass, but during wet seasons the needlegrass is more conspicuous. By overgrazing, the mountain sage comes in and in time will become one of the dominant species of the type. This type is most important in portions of North and South Dakota and eastern Montana, on level land having a rather loose sandy black loam soil. The rainfall over the area occupied by this type ranges from 15 to 18 inches and moistens the soil to a depth of 14 to 18 inches. Agriculturally, lands of this character usually produce good crops of grains except during years of less than average moisture supply. They also afford very good grazing, ranging from 40 to 80 head of cattle per section in most areas. The western needlegrass, however, is not readily grazed after it matures, owing to its tough texture.

(13) GRAMA-BUFFALO GRASS (*Bulbilis dactyloides* (Nutt.) Raf.).—The grama-buffalo grass type, shown by Plate 3, A, which is composed of a mixture of grama and buffalo grasses, is one of the most extensive types on the Great Plains south of the Montana line. It grows on hard or tight land where the soil moisture only rarely penetrates below the second foot and crop failures are sure during years of less than average moisture supply. Corn and wheat are the chief crops in the North, while the grain sorghums are ever present in the South. The carrying capacity over most of the lands supporting this type ranges from 25 to 50 head of cattle per section.

(14) GRAMA-BUFFALO GRASS, AND WESTERN NEEDLEGRASS.—A mixed stand of grama grass, buffalo grass, and needlegrass makes up this type. The needlegrass is taller than the other grasses and is more conspicuous, especially in wet years. The type also usually contains a scattering of Psoralea (*P. argophylla*), cone flower (*Echinacea*), and other flowering plants. Its soil and range are similar to Type (13). It is productive for wheat and corn in the North and grain sorghums in the South during years of average or more than average moisture supply. The carrying capacity of this type ranges from 35 to 75 head of cattle per section.

(15) GRAMA-BUFFALO, AND WIRE NEEDLEGRASS (*Aristida longiseta* Steud.).—This type, illustrated by Plate 3, B, is composed of an even cover of grama and buffalo grass, with scattered bunches of wire needlegrass. This is a common type on loose, sandy loam soils throughout the western Great Plains region. On heavy land this type occurs on the eastern edge of the high plains. Agriculturally, this land is productive of wheat, corn, and grain sorghums during years of average or more than average moisture supply. As grazing land, it will carry from 20 to 40 head of cattle per square mile. The wire needlegrass is too tough to be palatable when it matures.

(16) GRAMA-BUFFALO, AND WHEATGRASS.—Large areas of rather heavy soils, mostly clays and clay loams throughout the Great Plains region, especially

in western South Dakota, eastern Wyoming, and western Nebraska, are vegetated by this type. It consists of a sod of grama grass and buffalo grass and varying amounts of wheatgrass. In dry years the short grasses are more abundant, but in wet years the wheatgrass appears to be the dominant species. Considerable native hay is cut from lands containing this type. It also furnishes a large amount of grazing for range stock. The carrying capacity varies from 30 to 75 head of stock per section. The heavy soil makes crop production doubtful during dry years, but relatively heavy during years of excessive rainfall.

(17) GRAMA-BUFFALO, AND WILD ALFALFA.—The vegetation of this type is composed of an even sod of grama grass and buffalo grass over which plants of wild alfalfa are more or less equally scattered. The soil is similar to Type (9). This type does not occur to any appreciable extent north of Yellowstone River in Montana or in North Dakota. It indicates more than usual water supply, due either to precipitation or to flood water. The soil is deep as compared with Type (13), and it will often produce crops when the pure short grass land fails. The carrying capacity on most of the lands producing this type ranges from 30 to 65 head of cattle per section.

(18) GRAMA-BUFFALO GRASS, AND SOAPWEED YUCCA (*Yucca glauca* Nutt.).—The vegetative covering of this type is composed of an even stand of grama-buffalo, the latter usually predominating, with scattering bunches of soapweed yucca. It is very common in the southern Great Plains, growing on a loose, sandy loam soil. Land producing this type of vegetation is successfully used for growing corn and grain sorghums, and in some of the more favorable localities good yields of wheat are obtained in years of more than average precipitation. It provides nutritious and palatable forage, having a grazing capacity of 25 to 50 head of cattle per square mile.

(19) GRAMA-BUFFALO, AND MESQUITE (*Prosopis glandulosa* Torr.).—This is a very common type in the southern Great Plains of western Texas and eastern New Mexico, growing on hard lands. It is composed of an open sod of grama grass and buffalo grass, over which are scattering bunches of mesquite. Rainfall varies from 15 to 18 inches over the region occupied by this type. Moisture is insufficient, except in years of abnormal rainfall, to produce any cultivated crops other than sorghums. This type has a carrying capacity of 40 to 60 head of cattle per section.

(20) GRAMA-BUFFALO GRASS, AND MATCHWEED.—Plate 4, A, shows this type, which is made up of a sod of grama grass and buffalo grass over which is scattered matchweed in varying densities. The distribution is the same as Type (13). Where it occurs naturally it indicates land of doubtful agricultural value and grazing land of low carrying capacity, varying from 15 to 25 head of cattle per square mile. This type develops on land previously characterized by Type (13) as a result of overgrazing or breaking.

(21) WHEATGRASS.—This grass usually does not form a sod, but is found in open stands consisting of upright single plants scattered uniformly over the surface. It is widely distributed throughout the western United States, growing in heavy clay soils that usually contain alkali. It is very common in southeastern Montana and northwestern South Dakota, and it is of doubtful agricultural value, due mainly to the heavy soils in which it grows. In years of abnormal precipitation, however, high yields of grain have been obtained on lands producing this type. It is very palatable and nutritious to all classes of livestock, but because of the open stand and tendency to fail during dry years its carrynig capacity ranges as low as 10 head of cattle per section. The better areas will support 50 head per square mile.

(22) WIRE NEEDLEGRASS.—In addition to wire needlegrass, this type frequently contains patches of short grass and a scattering of flowering plants. It is a common

type in the Great Plains, growing in a rather loose sandy or gravelly loam soil. Toward the eastern side of the Great Plains it grows on a heavy soil. As agricultural land it is equal to, or possibly superior to, Type (15), which it resembles closely. Wire needlegrass has very little grazing value and is only taken by stock when other feed is scarce. However, the short grasses produced in the type afford fair grazing. The poorer gravelly lands producing this type have a low carrying capacity of not more than 15 head of cattle per section. The better lands, however, will graze double this number.

(23) NIGGERWOOL.—This type which is composed of a more or less pure stand of niggerwool occupies rather a limited area in the Great Plains section of Wyoming and Montana, growing usually on ridges or benches, where growing conditions are less favorable, due to shallow rocky soil or low rainfall. The land is of very little value for producing crops unless water is available for irrigation. Niggerwool is rather tough, and therefore has a low carrying capacity, 5 to 15 head of cattle per section.

(24) WESTERN NEEDLEGRASS.—Although needlegrass is widely distributed throughout the western United States, this type, which is composed of a medium stand of needlegrass containing scarcely any other plant species, has only been observed to any appreciable extent in northeastern Montana and northwestern North Dakota, where it occupies smooth to gently rolling bench lands, having a deep fertile black loam soil. This land is very fertile and easily tilled and since it occupies areas where the rainfall ranges from 15 to 18 inches, good crops of small grain can be produced in all but the dry years. This species affords fair forage when young but when it matures is too tough to be readily grazed by stock. Most of the tillable better lands that support this type are being successfully used for producing crops, mainly wheat.

(25) MATCHWEED.—This low-growing suffrutescent plant seldom occurs in pure stands except over burned areas or areas that have been greatly overgrazed or on old breakings. Its predominance on these locations is only temporary as it is gradually replaced, on the return to normal conditions, by the species originally growing on these lands. It is native, however, to rocky, gravelly areas, or on lands having very shallow soil where it is generally associated with a few other species. This type is very common in the southern Great Plains and in the Great Basin. The acreage occupied by its natural habitat is rather small, however, regardless of its common occurrence. It is worthless for grazing, which accounts partly for its invasion of overgrazed areas. In its natural habitat it indicates nonagricultural land unless irrigated.

(26) MATCHWEED AND MOUNTAIN SAGE (*Artemisia frigida* Willd.).—The matchweed and mountain sage type is found throughout the Great Plains on smooth bench lands having a rather coarse or gravelly loam soil. In the northern part of the Plains, mountain sage is more important and matchweed in the southern part. In the central Plain region this type indicates conditions intermediate between Type (25) in the South and Type (5) in the North. It consists of a mixed stand of matchweed and mountain sage growing over an open stand of short grass (grama or grama-buffalo), and characterizes old breakings, overgrazed land, and shallow soils where conditions are not favorable for a short grass cover. The type has low value for grazing.

(27) BLACK GRAMA (*Bouteloua eriopoda* Torr.).—The black grama type is composed of a relatively pure cover of black grama with scarcely any other species. It does not form a sod cover but branches out loosely to produce a relatively large amount of top growth. This species is dominant on large areas of grassland in the southwestern part of New Mexico and southeastern Arizona, where it grows in a light loam soil. It is of little value for growing crops except in good years when forage crops can be successfully produced. It is a very important range

grass, being highly palatable to all livestock. Carrying capacity ranges from 15 to 30 head of cattle per section, and in very favorable years, it makes sufficient growth on very favorable habitats to be cut for hay.

(28) Hairy Grama (*Bouteloua hirsuta* (Lag.)), Grama; and Sand Sage (*Artemisia filifolia* Torr.).—This type grows on sandy soils throughout the central Great Plains region, especially eastern Colorado, and is made up of an open stand of sand sage growing over an even sod composed mainly of hairy grama and grama. It has doubtful agricultural value except for sorghum and corn, since the soil is likely to blow if cultivated. As grazing land it will carry from 15 to 25 head of cattle per section. It will not stand heavy grazing owing to the loose texture of the soil. The sand sage also comes in as a result of overgrazing which decreases the grazing capacity of the range.

(29) Bunchgrass (*Andropogon scoparius* Michx.).—Lands on which an open stand of bunchgrass comprises most of the vegetation are classified under this type. It occurs on the sandhills and rough, rocky, or gravelly hills of the central or western high plains. It should not be confused with the relatively pure close stands of this grass on the loam soils of the eastern and southern high plains. The best type of this land is good corn or sorghum land, but in the western portion it often characterizes nonagricultural land. Bunchgrass when young is very palatable and nutritious to livestock and is also valued as winter range since it is not so easily covered with snow as the short grasses.

(30) Sand Hills Mixed.—Plate 4, B, illustrates a typical sand hills mixed type which consists of a mixture or alternation of sand sage, bunchgrass, big blue stem (*Andropogon furcatus* Muhl. or *Andropogon hallii* Hack.), sandgrass (*Calamovilfa longifolia* (Hook.) Scribn.), hairy grama (*Bouteloua hirsuta* Lag.), and frequently a number of flowering plants. It occurs on sand hills or rolling sandy land throughout the central and southern Great Plains, but is probably most common in the sand hills of eastern Colorado. This type of range supplies considerable forage and is especially valuable in dry years when little feed is produced on the hard lands. Its carrying capacity is from 20 to 50 head of cattle per section. As agricultural land it is subject to blowing and the whole area may be transformed by breaking into a blowout.

(31) Black Grama and Wire Needlegrass.—Lands having a uniform sod of black grama with varying quantities of wire needlegrass are listed under this type. The amount of wire needlegrass present in this type is usually governed by soil conditions, this species being more abundant on lands having coarse gravelly soils. A very common type in southwestern New Mexico and eastern Arizona, growing on slightly gravelly, rocky, or sandy loam soils. Areas producing this type can be used for growing forage crops only in favorable seasons. Black grama is excellent forage, but wire needlegrass is grazed only for a short time after growth starts. The carrying capacity of the black grama and wire needlegrass type ranges from 15 to 30 head of cattle per section.

(32) Black Grama and Tobosa (*Hilaria mutica* (Buckl.) Benth.).—This type is made up of varying quantities of tobosa grass growing in a relatively even stand of grass composed mainly of black grama. It usually grows in rather fine compact soils on flats that receive some flood water. The amount of tobosa grass in this type is directly proportionate to the amount of flood water received. This vegetative type occupies quite a large acreage in southwestern New Mexico and southeastern Arizona and possesses a farming and grazing value similar to Type (31).

(33) Tobosa Grass.—The tobosa grass type is applied to vegetative associations that are composed almost entirely of tobosa grass. This is the predominant type on flats and swales in New Mexico and eastern Arizona that are subject to flooding, where the soil is a fine compact loam or a clay loam. It occupies land

too arid to use for crop production unless additional moisture is available from flooding. The kind and amount of crops raised would depend upon the quantity of flood water. Tobosa grass is grazed when green but is practically worthless when dry.

(34) GALLETA (*Hilaria jamesii* (Torr.) Benth.).—Galleta grass is found in pure stands in this type. It is usually grazed rather closely so that it forms an even turf. This species is common in New Mexico and eastern Arizona but it occupies rather small areas. In Utah and Nevada, however, it occurs over rather large areas and it constitutes one of the best grazing types in the Great Basin. Very good grazing for all classes of livestock and can withstand heavy grazing. Usually more valuable for the native forage it provides than for growing cultivated crops. The carrying capacity varies considerably according to the density of the galleta. On most areas, however, the grazing capacity will range from 25 to 100 head of cattle per section.

(35) CROWFOOT GRAMA (*Bouteloua rothrockii* Vasey).—This species while found in combination with other species often forms pure stand and during wet years it makes sufficient growth on the more favorable habitats to cut for hay. Common in Arizona on mountain mesas and mountain foothills where it occupies a deep black loam soil. The most favorable habitats of this type can be used for producing cultivated forage crops. Crowfoot grama is highly palatable and nutritious but will not stand heavy grazing. It has a carrying capacity of from 20 to 35 head of cattle per section.

(36) *Hilaria rigida* Benth.—This grass resembles a shrub in its coarse upright growth. Its range is limited largely to the Colorado desert where it is frequently found in pure stands of rather scattered bunches. It develops best in areas where sand is slowly being deposited over the soil. It is almost too coarse to be readily taken by stock but is one of the main perennial grazing plants in the desert regions, so that it affords considerable feed. The habitat of this species is too arid to produce any cultivated crop without irrigation. It has a carrying capacity of from 2 to 10 head of cattle per section.

(37) TUSSOCK GRASS (*Sporobolus airoides* Torr.).—Tussock grass, illustrated in Plate 5, A, is a rather coarse-tufted grass forming a big bunched growth often a foot in diameter at the base. It is common along water courses on rather heavy alkaline or "gyp" soils in the southern Great Basin, New Mexico, and Arizona, and adjacent portions of the High Plains. Readily grazed by cattle and horses when green. It is most abundant on aklali land that receives some flood water. Lands occupied by this type usually contain too much alkali to be used for growing crops. It affords the best grazing of any of the alkali grasses, having a carrying capacity of from 50 to 100 head of cattle per section.

(38) SACATON (*Sporobolus wrightii* Munro).—Sacaton grows in tall coarse bunches forming a dense bunchlike stand on deep alluvial land relatively free from harmful amounts of alkali. It therefore characterizes land excellent for irrigation. It is found in eastern Arizona, New Mexico, and western Texas. Sacaton makes good hay, especially for horses. It is fairly good for grazing but is easily killed out by excessive tramping. It has a carrying capacity of from 50 to 100 head of cattle per section.

(39) WHEAT BUNCHGRASS (*Agropyron spicatum* (Pursh) Rydb.).—The wheat bunchgrass type illustrated by Plate 5, B, is applied to open grass lands composed almost entirely of wheat bunchgrass. This is the dominant type in the Columbia River Basin in Washington and below the timber zone in northeastern and north-central Oregon. It is also common in the rolling foothills and lower plateaus of Idaho and northern Utah, growing on well-drained loamy soils. Most of the best wheat lands of Oregon and Washington were originally vegetated by this type, so where it is growing in dense thrifty stands it is indicative of

favorable possibilities for grain production. It affords very good grazing, especially for cattle and horses, and has a carrying capacity ranging from 25 to 60 head of cattle per section.

(40) CURLY BUNCHGRASS (*Festuca ovina* L.).—Curly bunchgrass is a very common type on rolling foothills of eastern Idaho, western Colorado, and northern Utah, growing in deep rather coarse loam soil. It is frequently associated with Junegrass (*Koeleria cristata* (L.) Pers.), blue grasses (Poa), and Stipa. It frequently merges into the sage-brush type, where it is the dominant herbaceous species. The moisture requirements of this type are such that the lands it occupies can be successfully used for the production of wheat and oats, the main limitation being the length of growing season, which is frequently too short to mature grains. It affords excellent grazing, especially for cattle and horses, having a carrying capacity of from 30 to 60 head of cattle per section.

(41) MIXED MOUNTAIN GRASS.—A mixed stand of grasses composed mainly of bluegrass (Poa), curly bunchgrass, Junegrass, wheat bunchgrass, and needlegrass (Stipa), make up this type. About one-third of the stand of vegetation may also be composed of weeds, principally wild geranium (*Geranium incisum* Nutt.), alum root (*Sieversia ciliata* (Pursh) G. Don.), balsam root (*Balsamorrhiza sagittata* (Pursh) Nutt.), lupines, sunflower (Helianthella species), and Senecios. This type is common on rolling foothills and lower mountain lands of Colorado, Utah, and Idaho, usually occupying well-drained deep loam soils. The growing season is usually too short to mature cultivated crops, but this type furnishes excellent grazing, having a carrying capacity that ranges from 50 to 100 head of cattle per section for a four to five months grazing season.

(42) ARIZONA FESCUE (*Festuca arizonica* Vasey).—Grass lands where *Festuca arizonica* is the predominant species are classified in this type. Its range is confined to foothill and mountain areas above 6,000 feet in Arizona and New Mexico. It is the most comon mountain bunchgrass in the Southwest, and is frequently the predominant herbaceous species in open stands of yellow pine timber. As agricultural land it is capable of crop production without irrigation during exceptionally good years. The growth season is best adapted to small grains. It affords excellent forage during its early season of growth, but on maturity it becomes too coarse to be grazed. It has a carrying capacity which ranges from 20 to 30 head of stock per section.

(43) SHORT-LIVED GRASS TYPE.—This type consists of a mixture of small short-lived grasses. The following are often prominent: *Scleropogon brevifolius* Phil., *Aristida bromoides* H. B. K., *Bouteloua aristoides* (H. B. K.) Griseb., and short-season annuals. It is widely distributed in the Southwest, growing on mesas and foothills where desert or semidesert conditions prevail in a loamy soil, sometimes slightly sandy. This type usually marks badly overgrazed areas and is not therefore indicative of the character of the soil or potential grazing value.

(44) GIANT WILD-RYE (*Elymus condensatus* Presl).—This species, illustrated in Plate 6, A, grows in a deep rich alluvial soil free of harmful quantities of alkali. It is also common in waste places, such as along ditch banks. It is widely distributed throughout the Northwestern States, found usually in small patches and in isolated bunches, and is very conspicuous due to its tall bunchy growth, but the area it occupies is rather small. It marks land unusually valuable when placed under irrigation and is of very little grazing value owing to its coarse texture.

(45) WEED GRASS.—This type is limited chiefly to California where it is the principal type on the valley floors and lower foothills on lands containing little or no alkali. It consists of a more or less even stand of annuals or short-lived grasses, chief among which are three species of brome grass (Bromus species), wild oats (*Avena fatua* L.), and filare, also known as alfilaria, (*Erodium cicutarium* (L.) L'Hér.). In Washington and Oregon a similar type occurs on overgrazed

lands. It indicates lands from which the original vegetation has been driven out by overgrazing. In California this was done during early Spanish days. Although it has little indicative significance from the standpoint of crop production, much of the land characterized by this type is capable of producing crops of wheat and other small grains without irrigation. It supplies practically all the feed in the unimproved pastures and ranges in the valleys and lower foothills of California. The carrying capacity is from 15 to 30 head of cattle per section.

(46) WET MEADOW.—Classified in this type are all areas that remain wet or moist throughout the summer, where sedges and rushes are the dominant species of vegetation. These lands are usually located in the mountain valleys and are quite generally used for cutting wild hay. They have a rich black peaty soil, which, if drained, makes very good farm lands, but they are usually located at elevations too high for grains to mature. These lands afford very good grazing, carrying capacity ranging from 100 to 300 head of cattle per section, and are located throughout the mountainous areas of Colorado, Utah, and the Northwest.

(47) DRY MEADOW.—Open parks that are wet during the early part of the summer, but become moderately dry during the latter part of the season are included in this type. It includes the "Camas Prairies," so common in Idaho, and is composed of a mixture of weeds and grasses. The weeds are confined mainly to common camas (*Quamasia quamash* (Pursh) Coville), white flowered dock (*Polygonum*, Wyethia sp.), while the grasses are principally red top and other species of Agrostis. The growing season is usually too short for maturing cultivated farm crops. However, it supplies good grazing, having a carrying capacity ranging from 50 to 100 head of cattle per section. Its range is Colorado and the Northwest.

(48) MOUNTAIN WEED.—This type includes all untimbered mountain lands where nongrasslike species of herbaceous vegetation predominate. The principal species found in this type are wild geraniums, lupines, Senecio, white clover, dandelions, tall larkspurs, umbellifers, and Potentillas. This type is very common in moist rich soils in the higher mountainous areas in Colorado and the Northwest. It has not been utilized for crop production, mainly on account of the short growing season, but provides excellent summer range for sheep, having a carrying capacity of from 300 to 600 head of sheep per section.

(49) SAGEBRUSH (*Artemisia tridentata* Nutt.).—The sagebrush type illustrated by Plate 6, B, consists of a miniature forest or scattered brush land of sagebrush. Plants vary in height from 1 to 7 feet. This is the most common shrub throughout the Great Basin, growing in well-drained loamy soils. The height and abundance of plants are governed by the depth, quality, and moisture content of the soil. A dense stand of tall sagebrush is indicative of very favorable possibilities for the production of small grains without irrigation. It represents the best type of land in the Great Basin for either arid or irrigated farming. This type is grazed by stock, mainly sheep, during fall and winter when little or no herbaceous feed is available.

(50) SMALL SAGEBRUSH (*Artemisia nova* A. Nels.).—Plate 7, A, illustrates this type, which is a lower growing sagebrush than the preceding species, ranging in height from 1 to 3 feet and presenting during the growing season a more yellowish color than the sagebrush. The range of this plant is similar to that of sagebrush, but it occupies a rather limited area on rocky or shallow soils. As agricultural land this type is far inferior to a cover of pure sagebrush. The grazing value is rather low, 5 to 10 head of cattle per section.

(51) SCAB SAGEBRUSH (*Artemisia rigida* (Nutt.) A. Gray).—Lands containing a comparatively even stand of scab sagebrush, a species ranging from 1 to 2 feet

high, having twisted gnarled stems, and a comparatively small amount of foliage, are classified in this type. It occurs principally on the scablands in Washington and Oregon, growing on rocky soil, wholly unsuited for crop production. The grazing value is poor, less than 10 head of cattle per section.

(52) Hop-Sage (*Grayia spinosa* (Hook.) Moq.).—Hop-sage resembles sagebrush when not in leaf, but has thick fleshy leaves which contrast sharply with the thin silvery leaves of the sagebrush. It is called "hop-sage" because of the character of the fruits which resemble hops. This species is very abundant and has a wide range throughout the very arid sections of the West, particularly Nevada and western Utah. It is also found in the Red Desert of Wyoming, growing with shadscale and sagebrush. Land characterized by this type is of little or no value for crop production unless irrigated. It is of little forage value except as winter range for sheep.

(53) Antelope-brush (*Purshia tridentata* (Pursh) DC.).—The antelope-brush type, shown by Plate 7, B, is dominant in sandy soils along the Columbia River in Washington and Oregon. It also occurs through the Great Basin region as well as in California. As a rule it is associated with sagebrush. Together they usually characterize land more rocky and therefore less suited to crop production than pure sagebrush. They supply a fair browse for cattle and sheep, having a carrying capacity of 20 to 30 head of cattle per section.

(54) Shadscale (*Atriplex confertifolia* (Torr.) S. Wats.).—Plate 8, A, illustrates a typical shadscale area which supports scattered even stands of shadscale, the plants ranging from 6 inches to 2 feet in height and usually presenting a uniform rounded appearance. The leaves are leathery and seeds are produced in abundance. Plants are spiny and too rigid to be moved by an ordinary wind. This is a very common and widely distributed type in the Great Basin, growing frequently in rather heavy alkaline soils. In most localities it characterizes a shallow soil with alkali in the second foot and also to a lesser extent in the first foot. It is nonagricultural unless irrigated and drained to remove excess alkaline salts. It is one of the main plants grazed by sheep on the winter ranges. It has a carrying capacity of 25 to 100 head of sheep per section for a short intermittent grazing period.

(55) Salt Sage (*Atriplex corrugata* S. Wats. and *A. nuttallii* S. Wats.).—This species, also known as saltbush, illustrated by Plate 8, B, grows in low mats varying in size from a few inches to several feet across and seldom exceeding 6 inches in height. The mats are often scattered over an otherwise barren surface. It is found in rather heavy saline soils in southwestern Wyoming, west-central Colorado, east-central and western Utah, and Nevada. Readily browsed by sheep during the winter season. Growth occurs very early in the season, and it is then readily grazed by sheep. It has a carrying capacity of 25 to 75 head of sheep per section. This species indicates nonagricultural land, owing mainly to the high alkali content of the soil.

(56) Little Rabbitbrush (*Chrysothamnus stenophyllus* (A. Gray) Greene).—This type, illustrated by Plate 9, A, is applied to lands containing a rather even growth of small bunches, or of scattered larger bunches, of little rabbitbrush. Flowers are produced in great quantity and the area presents a uniform bright yellow color during the flowering period. The bushes are often as much as 2 feet high, but usually much smaller. It is indigenous to Utah and Nevada on light soils free from alkali. This species very frequently comes in on burned sagebrush and shadscale land. It has little value as grazing land.

(57) Rabbitbrush (*Chrysothamnus graveolens* (Nutt.) Greene), and Tussock Grass.—Lands containing a fairly even or occasionally matlike sod of tussock grass over which are scattered plants of rabbitbrush are classified in this type. Areas supporting this type have too much alkali for growing cultivated crops.

As a rule, part of its moisture supply comes from flood water. It is good grazing land but nonagricultural, except with drainage and careful irrigation. It has a carrying capacity of 20 to 40 head of cattle per section.

(58) WINTER-FAT (*Eurotia lanata* (Pursh.) Moq.).—A representative winter-fat type is shown on Plate 9, B, consisting of a scattering growth of winter-fat with little or no other species of vegetation. The winter-fat plants are very white and hairy and range in height from 3 to 12 inches and occasionally more. On account of its white appearance it is sometimes called white sage. This species grows on rather heavy soils in the more arid sections of the West, where it occupies rather a large area. It indicates land unsuitable for agriculture unless irrigated. Winter-fat is especially palatable and nutritious to sheep and supplies considerable forage on the winter ranges. In the Great Basin it is probably the best grazing land to be found in the desert regions.

(59) WHITE SAGE, also known as GREEN MOLLY (*Kochia americana* S. Wats.).—Lands are classified in this type which contain an open to scattering growth of white sage, a low plant, seldom exceeding 6 inches in height, having hairy round leaves that are more fleshy than those of winter-fat. It resembles winter-fat in general appearance, although it is not grazed to any appreciable extent. This type is not widely distributed throughout western Utah and Nevada, growing in heavy clay-loam soils. It is of doubtful agricultural value and of little grazing value.

(60) COLEOGYNE (*Coleogyne ramosissima* Torr.).—This type, illustrated by Plate 10, A, consists of an even growth of Coleogyne and a scattering of cliff-rose (*Cowania stansburiana* Torr), shrub buckwheat (*Eriogonum fasciculatum*), and tomatilla (*Lycium pallidum* (Miers). A very common type in the desert area of northwestern Arizona, southeastern California, southwestern Utah, and southern Nevada, where it occupies a region between that characterized by creosotebush and sagebrush. It usually grows in dry, porous desert soils. The land is of no value for crop production unless irrigated, when it is very valuable owing to the long growing season of the region it occupies. Coleogyne has no value for forage, so the type has a very low carrying capacity for livestock.

(61) GREASEWOOD (*Sarcobatus vermiculatus* (Hook.) Torr.).—This species, shown by Plate 10,B, is limited to subirrigated alkali lands throughout the West. The leaves of this plant are fleshy and the stems woody and spiny, ranging in height from 1 to 5 feet. The leaves are bright green in color and have a salty taste. This plant is a good indicator of lands having a rather heavy concentration of alkali. Under the proper system of irrigation land of this type will produce good crops, but unless drainage is supplied it is likely to develop excess quantities of alkali. The young branches of greasewood are readily grazed by sheep and cattle, especially during the winter.

(62) GREASEWOOD-SHADSCALE.—The greasewood-shadscale type consists of an even scattered growth of shadscale with an occasional scattering of plants of greasewood. It occurs on rather heavy alkaline soils in the Great Basin region, especially in Utah and Nevada. Under irrigation it can become productive if drained, but without irrigation it is worthless for crop production. It is relatively poor grazing land, mainly valuable as winter range for sheep, with a carrying capacity of 25 to 75 head of sheep per section.

(63) GREASEWOOD, SALT SAGE.—This type is commonly found in comparatively heavy alkali lands throughout western Wyoming and the Great Basin. It is composed of a very even scattered growth of salt sage over which at rather wide intervals occur large plants of greasewood. Its agricultural possibilities are similar to Type (55) but it has a larger amount of moisture penetration or possibly subsoil moisture. The grazing value is similar to the preceding type.

(64) GREASEWOOD, SALTGRASS (*Distichlis spicata* (L.) Greene).—Areas containing scattered plants of greasewood on a sod formed by saltgrass are classified

in this type. Saltgrass usually has a salty taste and is low-growing, seldom exceeding 8 inches in height. A very common type on alkali lands in the Great Basin region. Owing to excessive amounts of alkali found in the soil, it is nonagricultural. The type affords relatively good grazing land for cattle and horses, however. The carrying capacity is dependent upon the density of the saltgrass; 10 to 40 head of cattle per section.

(65) SALTGRASS.—This type, illustrated by Plate 11, A, occupies a large area of land in the Great Basin and even in portions of the Great Plains that have a shallow water table and contain a large alkali content. It may also contain barren alkali spots, the percentage of the surface occupied by these spots being governed by the concentration of alkali in the soil. Lands producing this type are nonagricultural unless the excess alkali is drained out. Palatable to cattle and horses until it matures when it is too tough for forage. It will stand very close grazing without injury and has a carrying capacity of 50 to 150 head of cattle per section.

(66) PICKLEWEED[3] (*Allenrolfea occidentalis* (S. Wats.) Kuntze).—The pickleweed type illustrated by Plate 11, B, contains a scattered growth of small plants or widely spaced larger plants of pickleweed. While the basal portion of this plant may be large and woody, the upper stems are thick and juicy and easily crushed in the hand. The fleshy stems are about one-eighth of an inch in diameter. Pickleweed grows on very alkaline soils in the Great Basin region. It has no grazing value and the land it occupies is nonagricultural.

(67) SEEPWEED (*Dondia moquinii* (Torr.) A. Nels.).—Seepweed is a common plant in the Great Basin, Colorado Desert, and adjacent valleys. It usually grows in rather dense pure stands in heavy alkaline soils. The branches of seepweed are very small and delicate and the leaves fleshy and easily crushed in the hand. The flowering branches often appear dark near the ends. It is worthless for agriculture and grazing.

(68) SAMPHIRE (*Salicornia utahensis* Tidestrom and *Salicornia rubra* A. Nels.).—This type occurs only under extremely saline conditions. The plants are very fleshy, seldom exceeding 6 inches in height, and have a very salty taste. It can be distinguished from pickleweed by the opposite leaves and branches. There are two types, the annual form (*Salicornia rubra*) and the perennial form (*Salicornia utahensis*). The former is usually much branched, the latter having as a rule only one or two branches. It grows in rather restricted areas throughout the Great Basin. It is of no value for grazing and characterizes nonagricultural land.

(69) CREOSOTEBUSH (*Covillea glutinosa* (Englem) Rydb.—Plate 12, A, shows an average tract of land vegetated by the creosotebush type, which consists of widely spaced plants of creosotebush growing either on barren soil or soil covered by winter or summer annuals, such as plantain, etc. This plant is often locally known as greasewood and is the most common shrub in the desert sections of southeastern California, southern Nevada, and western Arizona, where it grows in gravelly well-drained soil. Farther east where rainfall is more abundant it occurs on very shallow soils, soils with caliche near the surface. This is especially true in southeastern Arizona, southern New Mexico, and southwestern Texas. It is nonagricultural unless irrigated, and very poor for grazing, having a carrying capacity of less than 10 head of cattle per section.

(70) CREOSOTEBUSH AND BUR-SAGE (*Franseria deltoidea* Torr., *F. dumosa* A. Gray).—The creosotebush and bur-sage type, illustrated by Plate 12, B, is composed of an open to scattering stand of creosotebush with about an equal amount of two species of bur-sage. This type also frequently contains a scattering of paloverde, catsclaw, ironwood, mesquite, and giant cactus (*Carnegiea gigantea* (Engelm.)). It is dominant in broad valleys of western Arizona

[3] This is also known as burro-weed.

and southeastern California, where the mean annual rainfall is less than 10 inches. This type is nonagricultural unless irrigated. Neither creosote bush nor bursage has any grazing value. In wet years, however, annuals appear on the range occupied by this type which afford a limited amount of feed for stock. It has a carrying capacity of 0 to 10 head of cattle per section.

(71) ENCELIA (*Encelia farinosa* A. Gray) and CALIFORNIA SAGEBRUSH (*Artemisia californica* Less.).—This is a very common type in the dry inland valleys of California. It consists of a shrubby growth of *Encelia farinosa*, a rather coarse-stemmed plant with sunflowerlike flowers bearing rigid, white, and light-colored leaves; and *Artemisia californica*, a plant of similar size with very finely divided leaves and darker appearance. These shrubs have no value for grazing and the lands they occupy have no value for crop production, unless irrigated.

(72) SHRUB BUCKWHEAT (*Eriogonum fasciculatum* Benth.).—Shrub buckwheat is the dominant species in this type. It also contains scattering plants of Joshua tree (*Yucca arborescens*). With this type are often mixed a large number of shrub plants and occasionally juniper and creosotebush. This type is found in the desert regions of southeastern California and southern Nevada; its agricultural and grazing value are similar to the preceding type.

(73) DESERT BRUSH TYPE: CREOSOTEBUSH, IRONWOOD (*Olneya tesota* A. Gray), MESQUITE, PALOVERDE (*Parkinsonia microphylla* Torr.), and CATSCLAW (*Acacia greggii* A. Gray).—This is a mixed desert brush type in which creosotebush, mesquite, paloverde, ironwood, and catsclaw are the five important species. It is common in the desert region occupying large portions of western Arizona and southeastern California. It usually occurs along drainage channels. The aridity of the region where this type is found precludes the possibility of producing crops without irrigation. Mesquite and paloverde are the only species that have any appreciable grazing value. It has a carrying capacity of less than 10 head of cattle per section.

(74) CREOSOTEBUSH-DESERT GRASS.—A typical creosotebush-desert grass area is illustrated by Plate 13, A. It is composed of a scattered growth of creosotebush over grass land consisting usually of *Bouteloua rothrockii*, *Bouteloua eriopoda*, and *Hilaria mutica*. This type grows on loamy soils in southern New Mexico and Arizona. With the exception of *Hilaria mutica*, all the grasses growing in this type are highly palatable. It has a carrying capacity of 10 to 20 head of cattle per section. The land producing these species is too arid to grow crops unless irrigated.

(75) CREOSOTEBUSH AND CACTI (Opuntia species.)—This type grows in rather broken or rocky areas in southern New Mexico and the desert regions of Arizona, southern Nevada, and southeastern California. It is made up of a scattered growth of creosote bush and an abundance of cacti, especially the barrel cactus which varies in diameter from 1 to 1½ feet, and in height from 2 to 4 feet; and the round-stemmed Opuntias, especially the dense spiny *Opuntia bigelovii*. It is nonagricultural unless irrigated and of little or no value for grazing.

(76) CREOSOTEBUSH-OCOTILLO (*Fouquieria splendens* Engelm.)—The creosotebush-ocotillo type, which comprises a scattered growth of creosotebush and ocotillo, and many other plants of less conspicuous nature, occupies rocky and gravelly soils. It is widely distributed in the desert areas from southeastern California to western Texas. Both of the dominant species in this type are unpalatable to stock and the rocky nature of the habitat precludes the production of annuals. Lands producing this type are therefore nonagricultural and have a very low carrying capacity.

(77) GIANT CACTUS (*Carnegiea gigantea* (Engelm.) Britton & Rose), and BUR-SAGE.—Giant cactus and bur-sage are the dominant species in this type.

The former grows to a height of from 20 to 30 feet and is characterized by white straight spines. The type occurs in the desert regions of Arizona and California, where it is most frequently found on rocky ridges and foothills. It is of little or no grazing value and nonagricultural.

(78) MESQUITE.—Areas supporting little or no vegetative growth other than mesquite are classified in this type. This shrub, illustrated by Plate 13, B, varies in size from only a few feet to trees 30 feet high. It occupies a large acreage in southern New Mexico and Arizona and has considerable economic value for the stock feed supplied from the leaves and beans. The roots and the thicker stems also supply considerable fuel. Very common in deep fertile soil along drainage channels where subirrigation is usually received. The better types of this land are capable of producing forage crops such as sorghums, corn, and millet, during very favorable years. It has a carrying capacity of 5 to 15 head of cattle per section.

(79) MESQUITE AND CHAMISO (*Atriplex canescens* (Pursh) Nutt.).—All lands supporting a scattered growth of mesquite and chamiso, a shrub varying in height from 2 to 4 feet, are listed under this type. It is a very common type in southern New Mexico and Arizona, where it grows in clay loam soils. The mesquite trees are probably supported to a large extent by subsoil moisture. The agricultural possibilities of lands supporting this type are similar to those producing the preceding type. Chamiso is browsed extensively by cattle and sheep, making the type rather valuable for grazing. It has a carrying capacity of 20 to 30 head of cattle per section.

(80) MESQUITE AND MESQUITE GRASS.—The vegetation in this type consists of a scattered growth of mesquite over grassland which is usually composed of crowfoot grama, black grama, or grama-buffalo grass in the transition zone to this type, and in Texas buffalo curly mesquite (*Hilaria cenchroides* H. B. K.). It is usually characteristic of deep fertile loam soils. It is valuable grazing land, and where the grasses are mostly buffalo and curly mesquite, crops of cotton can be produced during the most favorable years. It has a carrying capacity of 20 to 30 head of cattle per section.

(81) *Parosela arborescens* (Torr.) Heller, and *Lepidospartum squamatum* A. Gray.—Parosela is a shrub from 3 to 6 feet high, with finely divided long leaves, the whole shrub presenting a misty or gray appearance and having deep indigo flowers. *Lepidospartum squamatum* is a rather low-growing shrub, harsh to the touch. This type is limited to the dry washes in southern Nevada and southeastern California and adjacent regions. Both species are worthless for grazing, and owing to the aridity of the region where they grow, crops can not be raised without irrigation.

(82) PLUCHEA (*Pluchea sericea* (Nutt.) Coville).—Pluchea is a tall straight-stemmed shrub, that seldom branches and grows in rather dense thickets. It was used to a considerable extent by the Indians to make arrows. This species occurs on strongly alkaline soil in limited quantities in western Arizona and southeastern California. It characterizes lands worthless for crop production and grazing.

(83) *Atriplex lentiformis* (Torr.) S. Wats.—*Atriplex lentiformis* is restricted to the low wet alkali lands of southeastern California, bordering salt lakes, particularly Salton Sink. It grows in large hummocklike clumps 6 to 15 feet across. There is usually little growth between these plants, which are widely spaced. Lands producing this type are incapable of crop production without leaching out the excess amount of alkali. It is grazed by stock mainly during periods of drought when little forage is available.

(84) DESERT SAGE (*Atriplex polycarpa* S. Wats.).—Desert sage, which is also known as saltbush and desert saltbush, illustrated by Plate 14, A, is limited largely to southeastern California, western Arizona, and southern Nevada.

It is a gray bush ranging from 2 to 5 feet in height, found mainly in chalky soils that contain more or less alkali. Dominant type on the bottom lands along Gila River, where it is found mainly in open stands. The type characterizes the most productive land of this region when it is placed under irrigation. It has a grazing value similar to that of *Atriplex lentiformis*.

(85) YUCCA DESERT GRASS.—Areas containing a scattered growth of yucca, usually *Yucca radiosa* (Englem.) Trel, over a grass cover composed usually of tobosa grass, are classified in this type. Yucca may also be distributed over areas characterized by *Bouteloua rothrockii* or *Bouteloua eriopoda*. It is common in New Mexico and eastern Arizona where it grows in rather loose open soils. Very little value for crop production without irrigation. The grasses in this type are palatable to livestock and the yucca is used to some extent as an emergency stock feed during droughts. The carrying capacity of this type is 15 to 25 head per section.

(86) BLACK BRUSH (*Flourensia cernua* DC.).—The black brush type contains few if any other species of vegetation. It grows in rather dense stands in well-drained porous soils in southern New Mexico and eastern Arizona. It has no value for crop production unless irrigated. It is also nonpalatable to all classes of livestock.

(87) BLACK BRUSH GRASS.—Plate 14, B, illustrates a typical area supporting this type, which is made up of a scattered stand of black brush and a little creosotebush with varying densities of grasses, consisting mainly of tobosa, black grama, and false needlegrass (*Scleropogon brevifolius* Phil.). This type is common in the semidesert regions of southern New Mexico and eastern Arizona. It characterizes lands that have little or no value for growing crops. With the exception of tobosa, which is only grazed when green, the other grasses are highly palatable to all classes of livestock. It has a carrying capacity of 10 to 20 head of cattle per section.

(88) NOLINA (*Nolina microcarpa* S. Wats.).—Nolina, also known as bear grass, produces a dense tufted bunched growth having very coarse foliage, varying in height from 2 to 4 feet. It is common in rather dry rocky localities in southern New Mexico and Arizona. It is not grazed owing to its tough character, and the localities where it grows are usually too rocky to have any value for producing crops.

(89) SHINNERY (*Quercus havardii* Rydb.).—This type, which is illustrated by Plate 15, A, is very common over the sandhills of New Mexico, Texas, and probably in western Oklahoma. It consists largely of an even distribution of the low-growing shinnery, seldom exceeding 2 feet in height. It often contains a scattered growth of mesquite. Browsed to a limited extent by livestock. It is very important in years of drought when other feed is very scarce. It is reported to be poisonous to cattle when the leaves are budding out. Shinnery lands are too sandy to be used for crop production.

(90) WILLOW (*Salix sp.*).—This type is restricted to narrow strips along creeks or other wet or moist places in the mountainous regions of the West, at elevations where the growing season is frequently too short to mature grain crops. Willows are also frequently found in small patches in wet meadows. Unless the willows are very dense a good undergrowth of sedges, rushes, and grasses is produced which are very palatable to sheep and cattle and thus give the land a high carrying capacity.

(91) MOUNTAIN BRUSH.—This type, shown by Plate 15, B, includes all brush land where chokecherry (*Prunus melanocarpa* (A. Nels.) Rydb.), snowberry (*Symphoricarpos* sp.), shadblow (*Amelanchier* sp.), oak brush (*Quercus utahensis* (A. DC.) Rydb.), scrub maple (*Acer* sp.), river hawthorn (*Crataegus rivularis* Nutt.), and sagebrush are the predominant shrubs, occurring either in equal quantities or with one predominating and with one or none of the

other species. Under the shrubs there is usually a good stand of grasses and weeds. This type is restricted to the mountain and foothill areas of the Rocky Mountains, about 6,000 feet elevation. With the exception of sagebrush, all the species are very good browse for cattle and sheep. Areas that can be cultivated are usually small and although lands of this character could be used for crop production their position and distribution are such that only small garden patches can be developed. The soil is usually dark and rich, but the growing season is sometimes too short to mature grains. The carrying capacity of this type is about 25 to 50 head of cattle per section.

(92) MOUNTAIN MAHOGANY (*Cercocarpus parvifolius* Nutt.).—Included in this type are all brush lands where mountain mahogany or closely related species predominate. It may be associated with oak brush, shadblow, river hawthorn, and mormon-tea. This type occupies drier soils than the preceding type. The stand of herbaceous vegetation under the shrubs is rather scattering. It occurs in rather low mountains and on foothills in Colorado, Utah and New Mexico. It is nonagricultural owing to the broken and rocky character of the surface but forms good browse for cattle and sheep, having a carrying capacity of 20 to 40 head of cattle per section.

(93) CHAPARRAL (*Ceanothus velutinus* Dougl.).—This is a very common brush type on burned-over timber lands, representing one of the stages of reproduction, where it often occurs in dense stands. It is also found on foothills associated with sagebrush, chokecherry, shadblow, aspen, or manzanita. It is a very common type above 6,000 feet in all the Western States except New Mexico and Arizona. It usually occupies lands too rough to be used for growing crops and it is also worthless for grazing.

(94) MANZANITA BRUSH TYPE.—This type includes all brush land where manzanitas (*Arctostaphylos manzanita* Parry, *A. pungens* H. B. K., *A. nevadensis* Parry) are the predominant shrubs. This type is very common in the mountain and foothill lands of Oregon and California, growing most commonly on southern slopes. In Oregon the manzanitas comprise 75 to 95 per cent of the shrubs in the type, while the remaining 5 to 25 per cent is made up of white oak (*Quercus garryana* Hook.), *Ceanothus cuneatus*, California laurel (*Arbutus menziesii* Pursh.) and black oak (*Quercus californica* (Torr.) Coop.). This type comes in on burned areas and is most frequently found in dense patches. Its agricultural possibilities are similar to the preceding type, and its carrying capacity is very low.

(95) SOUTHWEST MOUNTAIN BRUSH TYPE.—This is a mixed mountain and foothill brush type found in New Mexico and Arizona containing a predominance of one or all of the following shrubs: Catsclaw (*Acacia greggii* A. Gray), *Baccharis pteronioides* DC., small mesquite (*Mimosa biuncifera* Benth.), Apache plum (*Fallugia paradoxa* (D.Don) Endl.), mormon-tea (Ephedra), cliffrose, and shadblow (*Amelanchier* sp.). The last two species are usually found above 7,000 feet, while the others are found below this elevation. The lower species may have a scattering of scrub oak and mesquite. This is nonagricultural due to the broken and rocky character of the lands occupied by this type. It has a carrying capacity of 15 to 25 head of cattle per section.

(96) OAK BRUSH.—Classified under this type are all brush lands where oak brush or other closely related species predominate. This is a foothill type and is common in Utah, Colorado, New Mexico, and Arizona, where the oak brush consists mainly of *Quercus utahensis* (A.DC.) Rydb., and *Q. arizonica* Sarg. The latter species is restricted largely to Arizona and New Mexico. Associated with the oaks are the shrubs listed in Types (91) and (92) for Utah and Colorado and Type (95) for New Mexico and Arizona. In the coast region of northern California the oak brush type consists of a mixture of live oaks (*Quercus chrys-*

olepis Liebm., *Q. agrifolia* Nee, *Q. wislizenii* A.DC.) and scrub oak (*Quercus dumosa* Nutt.). This type grows mainly on foothill lands that are mainly nontillable, consequently it has little importance for growing crops. It has a carrying capacity of 20 head of cattle per square mile.

(97) CHAMISE. (*Adenostoma fasciculatum* Hook. & Arn.)—Chamise, also known as chamiso, illustrated by Plate 16, A, is a brush-land type found in the foothills and mountain regions of California and southern Oregon up to about 2,500 feet elevation. This type grows in dense stands on dry, rather rocky, slopes in the coastal ranges of California below the conifer timber zone. This species is worthless for grazing and it grows on lands that can not be used for crop production owing mainly to their rocky and broken character.

(98) *Ceanothus cuneatus* Nutt.—This type, shown by Plate 16, B, occupies the drier mountain slopes below the conifer timber zone on the inland mountain ranges of California and on the west slopes of the Cascades in southern Oregon. In Oregon, this species is associated with white oak (*Q. garryana* Hook), manzanita, and Garrya (*Garrya elliptica* Dougl.). Under the shrubs is a scattering of annual weeds and grasses. It is browsed quite readily by cattle, goats, and sheep.

(99) CALIFORNIA MIXED BRUSH TYPE.—This is a California brush-land type in which there is a mixture of chaparral and other brush species, no one standing out predominantly. In the coastal mountain region of northern California, this type consists of a mixture of chamise, *Ceanothus cuneatus* Nutt., live oak, scrub oak, blue brush (*Ceanothus integerrimus* Hook & Arn.), bitter cherry (*Prunus emarginata* (Dougl.) Walp.), white thorn (*Ceanothus cordulatus* Kellogg), mountain mahogany, poison oak (*Rhus integrifolia* (Nutt.) Benth. & Hook), and pepper wood (*Umbellularia californica* (Hook & Arn.) Nutt.). This type occupies the better sites where the soil and moisture are more favorable for plant growth than the previous type. It has only a low value for grazing and the surface is too rolling and mountainous to be used for cultivation.

(100) ASPEN (*Populus tremuloides* Michx.).—This type includes all lands where aspen makes up 70 per cent or more of the stand of tree species. It often occurs in pure stands, but is frequently found in mixture with various conifers. In Colorado and Wyoming the conifers are usually Englemann spruce, alpine fir, Douglas fir, or yellow pine. In Arizona and New Mexico they are mainly Englemann spruce, corkbark pine, alpine fir, Douglas fir, and white fir. In Idaho and Utah the conifer is usually Douglas fir, lodgepole pine, white fir, and Englemann spruce. Aspen makes good fence posts, and in central Utah it has been used quite extensively for mine props. There is usually enough herbaceous vegetation growing under the aspens to make it very good grazing, especially for sheep. The frost-free season is usually too short to mature cultivated crops.

(101) WOODLAND TIMBER.—This type includes all areas in woodland timber. The main species classified as woodland are piñon, digger pine, and juniper. Very common in the western United States, along the foothills on rather dry southern exposures particularly in the Southwest. Very little herbaceous growth is produced in this type, and it has a low grazing value. It is of little value for agriculture, except in the more favorable localities where forage crops can be produced.

(102) CONIFER TIMBER.—Classified in this type are all lands where conifer timber, except the species classified as woodland, is growing in sufficient quantities to have a conifer timber aspect. The value of conifer timber land for grazing varies considerably, the amount of feed produced being governed mainly by the density of the stand. Conifer timber in the West is also mainly located at elevations too high for the maturity of cultivated crops.

KEY TO AGRICULTURAL POSSIBILITIES OF VEGETATION TYPES

The natural vegetation as a rule does not indicate favorable or unfavorable topography or the tillable or untillable character of the soil. It does, however, serve as an index to the potential possibilities of the habitat for growing other plants. A few of the types described in this paper may cover a large area, representing a considerable range in growing conditions. Any existing difference will be shown, however, in the density and thrift of the stand of vegetation even where there are not any special plants present to indicate changes. On a large area of the range lands of the West, especially the unreserved public domain, overgrazing has changed the natural stand of vegetation to such an extent that the plants now growing on the land can not be used as an indicator of the agricultural possibilities. Overgrazed areas can be readily identified by the replacement of the more tender and succulent plants by tougher and non-palatable species and by the roots of the vegetation being exposed by tramping.

In the following key the types are listed roughly in order of the value of the lands they occupy for the production of cultivated crops. Since some of the types cover a wide range of growing conditions, they may be found on locations better or poorer than are indicated by this key.

The types are listed in two general classes: (1) Those indicating favorable possibilities for growing crops by dry farming, and (2) those indicating lands where cultivated crops can not be successfully produced by dry farming and are therefore mainly valuable for grazing. The types indicating favorable conditions for dry farming are also divided into two classes: (*a*) Those that indicate favorable possibilities for producing grain cereals, and (*b*) those indicating lands that are suitable for the production of forage crops. Each of these groups is further divided into three general classes—best, medium, and poorest types.

1. DRY FARM LAND.—Lands which under proper cultivation are capable of producing crops without irrigation more valuable than the natural vegetation.

(*a*) *Grain land.*—Types indicating lands suitable for the production of small grains.

Best types:

(11) Grama grass and valley sage.
(12) Grama grass and western needle-grass.
(24) Western needlegrass.
(5) Grama grass and mountain sage.
(7) Grama grass and sagebrush.
(9) Grama grass and wild alfalfa.
(91) Mountain brush land.
(40) Curly bunchgrass.

Medium types:

(42) Arizona fescue.
(39) Wheat bunchgrass.
(17) Grama-buffalo and wild alfalfa.
(47) Dry meadow.
(53) Antelope-brush.
(22) Wire needlegrass.
(13) Grama-buffalo grass.
(16) Grama-buffalo and wheatgrass.
(4) Grama grass, niggerwool, and Junegrass.
(45) Weed grass.
(15) Grama-buffalo and wire needle-grass.
(14) Grama-buffalo and western needle-grass.
(2) Grama grass and wheatgrass.
(96) Oak brush.

Poorest types:

(49) Sagebrush.
(21) Wheatgrass.
(3) Grama grass and niggerwool.
(56) Little rabbitbrush.
(1) Grama grass.
(44) Giant wild-rye.

(*b*) *Forage land.*—Lands which, under cultivation, are suitable in most localities for the production of forage crops, such as corn, sorghums, millet, cow peas, or small grains cut for forage.

Best types:

(29) Bunchgrass.
(100) Aspen.
(48) Mountain weed.
(41) Mixed mountain grass land.
(20) Grama-buffalo and matchweed.
(18) Grama-buffalo grass and soapweed yucca.
(90) Willows.
(28) Hairy grama, grama, and sand sage.
(19) Grama-buffalo and mesquite.
(80) Mesquite and mesquite grass.
(10) Grama grass and matchweed.

Medium types:

(30) Sand hills mixed.
(27) Black grama.
(8) Grama grass and chamiso.
(35) Crowfoot grama.
(95) Southwest mountain brush land.
(102) Conifer timber.
(101) Woodland timber.

Poorest types:

(89) Shinnery.
(32) Black grama and tobosa grass.
(33) Tobosa grass.
(31) Black grama and wire needlegrass.
(6) Grama grass, niggerwool, and sagebrush.
(23) Niggerwool.
(85) Yucca desert grass land.
(98) *Ceanothus cuneatus.*

2. Grazing lands.—Lands either unsuitable for the production of cultivated crops by arid farming or on which the production of native vegetation is more valuable than the crops that can be produced by arid farming.

Best types:

(46) Wet meadow.
(34) Galleta.
(38) Sacaton.
(37) Tussock grass.
(65) Saltgrass.
(64) Greasewood and saltgrass.
(57) Rabbitbrush and tussock grass.
(92) Mountain mahogany.
(58) Winter-fat.

Medium types:

(78) Mesquite.
(79) Mesquite and chamiso.
(54) Shadscale.
(55) Salt sage.
(74) Creosotebush-desert grass.
(87) Black brush grass land.
(62) Greasewood-shadscale.
(63) Greasewood-saltsage.
(99) California mixed brush type.
(73) Desert brush type.
(83) *Atriplex lentiformis.*
(61) Greasewood.
(52) Hop-sage.
(36) *Hilaria rigida.*

Poorest types:

(88) Nolina.
(50) Small sagebrush.
(51) Scab sagebrush.
(69) Creosotebush.
(97) Chamise.
(76) Creosotebush-ocotillo.
(75) Creosotebush and cacti.
(72) Shrub buckwheat.
(71) Encelia and California sagebrush.
(59) White sage.
(70) Creosotebush and bur-sage.
(66) Pickleweed.
(67) Seepweed.
(68) Samphire.
(82) Pluchea.
(81) *Parosela arborescens* and *Lepidospartum squamatum.*
(77) Giant cactus and bur-sage.
(60) Coleogyne.
(84) Desert sage.
(86) Black brush.

Types which do not indicate natural conditions, but come in as the result of cultivation, burning, or over-grazing:

(26) Matchweed and mountain sage.
(45) Weed grass.
(93) Chaparral.
(94) Manzanita brush type.
(25) Matchweed.
(43) Short-lived grass type.

KEY TO THE CARRYING CAPACITY OF THE VEGETATION TYPES

Practically all types of vegetation are used to some extent for grazing. Even in regions where sufficient moisture falls to produce crops and where the soils are suitable for this purpose, there is always a large acreage of physically non-tillable land that can be used only for grazing stock. In classifying the types of vegetation according to carrying capacity they have been listed in the approximate order of their carrying capacity in three groups: (1) Best, (2) medium, and (3) poor to worthless. The carrying capacity figures are intended to cover the bulk of the lands occupied by each type but there may be areas having greater or lower carrying capacities for some of the types than are listed here. The carrying capacities also vary from year to year depending upon growing conditions. The figures given in this paper represent the carrying capacity in an average year. The figures also represent the carrying capacity for the period of each year that the lands occupied by the types can be grazed without injury to the vegetation. Unless otherwise stated the grazing capacity figures apply to the number of cattle that can be grazed on a square mile or section of land.

Best Types

Vegetation type:	Carrying capacity
(46) Wet meadow	100–300
(90) Willows	75–150
(65) Saltgrass	50–150
(38) Sacaton	50–100
(37) Tussock grass	50–100
(48) Mountain weed (sheep)	300–600
(47) Dry meadow	50–100
(34) Galletagrass	25–100
(9) Grama grass and wild alfalfa	50– 90
(12) Grama grass and western needlegrass	40– 80
(19) Grama-buffalo and mesquite	40– 60
(14) Grama-buffalo and western needlegrass	35– 75
(16) Grama-buffalo and wheatgrass	30– 75
(41) Mixed mountain grass land	25– 75
(100) Aspen	30– 65
(17) Grama-buffalo and wild alfalfa	30– 65
(2) Grama grass and wheatgrass	30– 60
(11) Grama grass and valley sage	30– 60
(4) Grama grass, niggerwool, and Junegrass	30– 60
(40) Curly bunchgrass	30– 60
(39) Wheat bunchgrass	25– 60
(13) Grama-buffalo grass	25– 50
(5) Grama grass and mountain sage	25– 50
(18) Grama-buffalo grass and soapweed yucca	25– 50
(91) Mountain brush land	25– 50

Medium Types	Carrying capacity
(30) Sand hills mixed	20–50
(1) Grama grass	20–40
(24) Western needlegrass	20–40
(3) Grama grass and niggerwool	20–40
(57) Rabbitbrush and tussock grass	20–40
(15) Grama-buffalo and wire needlegrass	20–40
(29) Bunchgrass	20–40
(21) Wheatgrass	10–50
(92) Mountain mahogany	15–40
(35) Crowfoot grama	20–35
(42) Arizona fescue	20–30
(53) Antelopebrush	20–30
(7) Grama grass and sagebrush	20–30
(80) Mesquite and mesquite grass	20–30
(96) Oak brush	15–30
(45) Weed grass	15–30
(27) Black grama	15–30
(31) Black grama and wire needlegrass	15–30
(8) Grama grass and chamiso	15–30
(64) Greasewood-saltgrass	10–40
(102) Conifer timber	10–30
(10) Grama grass and matchweed	15–25
(22) Wire needlegrass	15–25
(28) Hairy grama, grama, and sand sage	15–25
(32) Black grama and tobosagrass	15–25
(85) Yucca desert grass land	15–25
(95) Southwest mountain brush land	15–25
(49) Sagebrush	15–25
(44) Giant wild-rye	15–25
(79) Mesquite and chamiso	10–30
(101) Woodland timber	10–25
(87) Black brush grass land	10–20
(74) Creosotebush-desert grass	10–20
(99) California mixed brush type	10–20
(89) Shinnery	10–20
(58) Winter-fat (sheep)	50–100
(54) Shadscale (sheep)	25–100
(55) Salt sage (sheep)	25–75
(62) Greasewood-shadscale (sheep)	25–75
(63) Greasewood-salt sage (sheep)	25–75
(33) Tobosa grass	10–20
(23) Niggerwool	5–15
(43) Short-lived grass type	5–15
(94) Manzanita brush type	5–15
(98) *Ceanothus cuneatus*	5–15
(10) Grama grass and matchweed	5–15
(26) Matchweed and mountain sage	5–15
(88) Nolina	5–15
(93) Chaparral	5–15
(56) Little rabbitbrush	5–15
(73) Desert brush type	5–12
(78) Mesquite	5–10
(50) Small sagebrush	5–10
(61) Greasewood (sheep)	25–50

Poor to Worthless Types	Carrying capacity
(36) *Hilaria rigida*	5–10
(25) Matchweed	5–10
(84) Desert-sage	10–
(51) Scab sagebrush	5–10
(69) Creosotebush	0–10
(86) Black brush	0–10
(75) Creosotebush and cacti	0–10
(70) Creosotebush and bur-sage	0–10

Little or No Grazing Value

(59) White sage.
(60) Coleogyne.
(66) Pickleweed.
(67) Seepweed.
(68) Samphire.
(71) Encelia and California sagebrush.
(72) Shrub buckwheat.
(76) Creosotebush-ocotillo.
(77) Giant cactus and bur-sage.
(81) *Parosela arborescens* and *Lepidospartum squamatum.*
(82) Pluchea.
(83) *Atriplex lentiformis.*

TYPES OF VEGETATION GROUPED BY REGION

The following is a list of the types of vegetation, giving their distribution in the regions outlined on the key map of the vegetation regions. The regions listed are believed to include practically the entire range for a large percentage of the types. A few, however, may extend to a limited extent into adjoining regions.

Type	Regions
(1) Grama grass (*Bouteloua gracilis*)	1, 2, 3, 7, 8
(2) Grama grass and wheatgrass (*Agropyron smithii*)	1, 2, 7
(3) Grama grass and niggerwool (*Carex filifolia*)	1, 2, 5, 7
(4) Grama grass, niggerwool, and Junegrass (*Koeleria cristata*)	1, 2, 7
(5) Grama grass and mountain sage (*Artemisia frigida*)	1, 2
(6) Grama grass, niggerwool, and sagebrush (*Artemisia tridentata*)	1, 5
(7) Grama grass and sagebrush	3, 5, 7
(8) Grama grass and chamiso (*Atriplex canescens*)	1, 2, 3, 5, 7
(9) Grama grass and wild alfalfa (*Psoralea tenuiflora*)	1, 2
(10) Grama grass and matchweed (*Gutierrezia sarothrae*)	1, 2, 3, 5, 7
(11) Grama grass and valley sage (*Artemisia nana*)	1
(12) Grama grass and western needlegrass (*Stipa comata*)	1
(13) Grama-buffalo grass (*Bulbilis dactyloides*)	1, 2
(14) Grama-buffalo grass and western needlegrass	1, 2
(15) Grama-buffalo grass and wire needlegrass (*Aristida longiseta*)	2
(16) Grama-buffalo and wheatgrass	1, 2
(17) Grama-buffalo and wild alfalfa	1, 2
(18) Grama-buffalo grass and soapweed yucca (*Yucca glauca*)	2
(19) Grama-buffalo and mesquite (*Prosopis glandulosa*)	2
(20) Grama-buffalo grass and matchweed	1, 2
(21) Wheatgrass	1
(22) Wire needlegrass	2, 7, 8
(23) Niggerwool	1, 5
(24) Western needlegrass	1, 2
(25) Matchweed	1, 2, 3, 7, 8
(26) Matchweed and mountain sage (*Artemisia frigida*)	1
(27) Black grama (*Bouteloua eriopoda*)	8
(28) Hairy grama (*Bouteloua hirsuta*), grama, and sand sage (*Artemisia filifolia*)	2
(29) Bunchgrass (*Andropogon scoparius*)	1, 2
(30) Sand hills mixed	1, 2

Type	Regions
(31) Black grama and wire needlegrass	8
(32) Black grama and tobosa grass (*Hilaria mutica*)	8
(33) Tobosa grass	2, 7, 8
(34) Galleta grass (*Hilaria jamesii*)	5, 6, 7, 8, 9
(35) Crowfoot grama (*Bouteloua rothrockii*)	7, 8
(36) *Hilaria rigida*	15
(37) Tussock grass (*Sporobolus airoides*)	2, 5, 6, 7, 8, 9, 15
(38) Sacaton (*Sporobolus wrightii*)	2, 6, 7, 8
(39) Wheat bunchgrass (*Agropyron spicatum*)	10
(40) Curly bunchgrass (*Festuca ovina*)	3, 10
(41) Mixed mountain grass	3, 10
(42) Arizona fescue (*Festuca arizonica*)	7
(43) Short-lived grass type	8
(44) Giant wild rye (*Elymus condensatus*)	3, 9
(45) Weed grass	12, 13, 14
(46) Wet meadow	3, 4, 7, 10, 11, 12, 14
(47) Dry meadow	3, 4, 10, 11, 12, 14
(48) Mountain weed	3, 4, 10, 11, 12
(49) Sagebrush (*Artemisia tridentata*)	3, 5, 6, 7, 9, 10, 11
(50) Small sagebrush (*Artemisia nova*)	3, 5, 6, 9
(51) Scab sagebrush (*Artemisia rigida*)	10
(52) Hop-sage (*Grayia spinosa*)	5, 6, 9
(53) Antelope-brush (*Purshia tridentata*)	3, 10
(54) Shadscale (*Atriplex confertifolia*)	5, 6, 9
(55) Salt sage (*Atriplex corrugata* and *A. nuttallii*)	5, 9
(56) Little rabbitbrush (*Chrysothamnus stenophyllus*)	9
(57) Rabbitbrush (*Chrysothamnus graveolens*) and tussock grass	2
(58) Winter-fat (*Eurotia lanata*)	5, 6, 9
(59) White sage (*Kochia americana*)	5, 9
(60) Coleogyne (*Coleogyne ramosissima*)	9, 15
(61) Greasewood (*Sarcobatus vermiculatus*)	5, 6, 8, 9
(62) Greasewood, Shadscale	5, 6, 9
(63) Greasewood, Salt sage	5, 6, 9
(64) Greasewood, Saltgrass (*Distichlis spicata*)	5, 6, 7, 9
(65) Saltgrass	1, 2, 5, 6, 7, 8, 9, 13, 15
(66) Pickleweed (*Allenrolfea occidentalis*)	8, 9, 15
(67) Seepweed (*Dondia moquinii*)	5, 6, 9
(68) Samphire (*Salicornia utahensis* and *S. rubra*)	5, 6, 9
(69) Creosotebush (*Covillea glutinosa*)	8, 15
(70) Creosotebush and bur-sage (*Franseria deltoidea, Franseria dumosa*)	15
(71) Encelia (*Encelia farinosa*) and California sagebrush	13, 15
(72) Shrub buckwheat (*Eriogonum fasciculatum*)	9, 15
(73) Desert-brush type: Creosotebush, ironwood (*Olneya tesota*), mesquite, paloverde (*Parkinsonia microphylla*), and catsclaw (*Acacia greggii*)	15
(74) Creosotebush-desert grass	2, 8
(75) Creosotebush and cacti (Opuntia species)	15
(76) Creosotebush-ocotillo (*Fouquieria splendens*)	8, 15
(77) Giant cactus (*Carnegiea gigantea*) and bur-sage	15
(78) Mesquite	2, 8, 15
(79) Mesquite and chamiso (*Atriplex canescens*)	8
(80) Mesquite and mesquite grass	8
(81) *Parosela arborescens* and *Lepidospartum squamatum*	15
(82) Pluchea (*Pluchea sericea*)	15
(83) *Atriplex lentiformis*	15

	Type	Regions
(84)	Desert-sage (*Atriplex polycarpa*)	15
(85)	Yucca desert grass	8
(86)	Black brush (*Flourensia cernua*)	8
(87)	Black brush grass	8
(88)	Nolina (*Nolina microcarpa*)	8
(89)	Shinnery (*Quercus havardii*)	2
(90)	Willow (Salix species)	3, 4, 7, 10, 11, 12, 13
(91)	Mountain brush	3, 10
(92)	Mountain mahogany (*Cercocarpus parvifolius*)	3, 7
(93)	Chaparral (*Ceanothus velutinus*)	3, 4, 11
(94)	Manzanita—brush type	12, 14
(95)	Southwest mountain brush	7, 8
(96)	Oak brush	3, 7, 8
(97)	Chamise (*Adenostoma fasciculatum*)	14
(98)	*Ceanothus cuneatus*	11, 12, 14
(99)	California mixed brush type	11, 12, 14
(100)	Aspen (*Populus tremuloides*)	1, 3, 4, 7
(101)	Woodland timber	1, 2, 3, 6, 7, 8, 9, 10, 11, 14
(102)	Conifer timber	1, 2, 3, 4, 5, 7, 8, 10, 11, 12, 14

DISTRIBUTION OF THE TYPES IN THE 15 VEGETATION REGIONS

Region 1

(1) Grama grass.
(2) Grama grass and wheatgrass.
(3) Grama grass and niggerwool.
(4) Grama grass, niggerwool, and June-grass.
(5) Grama grass and mountain sage.
(6) Grama grass, niggerwool, and sage-brush.
(8) Grama grass and chamiso.
(9) Grama grass and wild alfalfa.
(10) Grama grass and matchweed.
(11) Grama grass and valley sage.
(12) Grama grass and western needle-grass.
(13) Grama-buffalo grass.
(14) Grama-buffalo grass and western needlegrass.
(16) Grama-buffalo and wheatgrass.
(17) Grama-buffalo and wild alfalfa.
(20) Grama-buffalo grass and match-weed.
(21) Wheatgrass.
(23) Niggerwool.
(24) Western needlegrass.
(25) Matchweed.
(26) Matchweed and mountain sage.
(29) Bunchgrass.
(30) Sand hills mixed.
(65) Saltgrass.
(100) Aspen.
(101) Woodland timber.
(102) Conifer timber.

Region 2

(1) Grama grass.
(2) Grama grass and wheatgrass.
(3) Grama grass and niggerwool.
(4) Grama grass, niggerwool, and June-grass.
(5) Grama grass and mountain sage.
(8) Grama grass and chamiso.
(9) Grama grass and wild alfalfa.
(10) Grama grass and matchweed.
(13) Grama-buffalo grass.
(14) Grama-buffalo grass and western needlegrass.
(15) Grama-buffalo grass and wire needlegrass.
(16) Grama-buffalo and wheatgrass.
(17) Grama-buffalo and wild alfalfa.
(18) Grama-buffalo grass and soap-weed yucca.
(19) Grama-buffalo and mesquite.
(20) Grama-buffalo grass and match-weed.
(22) Wire needlegrass.
(24) Western needlegrass.
(25) Matchweed.
(28) Hairy grama, grama, and sand sage.
(29) Bunchgrass.
(30) Sand hills mixed.
(33) Tobosa grass.
(37) Tussock grass.
(38) Sacaton.
(57) Rabbitbrush and tussock grass.
(65) Saltgrass.
(74) Creosotebush-desert grass.
(78) Mesquite.
(89) Shinnery.
(101) Woodland timber.
(102) Conifer timber.

Region 3

(1) Grama grass.
(7) Grama grass and sagebrush.
(8) Grama grass and chamiso.
(10) Grama grass and matchweed.
(25) Matchweed.
(40) Curly bunchgrass.
(41) Mixed mountain grass.
(44) Giant wild-rye.
(46) Wet meadow.
(47) Dry meadow.
(48) Mountain weed.
(49) Sagebrush.
(50) Small sagebrush.
(53) Antelope-brush.
(90) Willow (Salix species).
(91) Mountain brush.
(92) Mountain mahogany.
(93) Chaparral.
(96) Oak brush.
(100) Aspen.
(101) Woodland timber.
(102) Conifer timber.

REGION 4

(46) Wet meadow.
(47) Dry meadow.
(48) Mountain weed.
(90) Willow (Salix species).
(93) Chaparral.
(100) Aspen.
(102) Conifer timber.

REGION 5

(3) Grama grass and niggerwool.
(6) Gramagrass, niggerwool, and sagebrush.
(7) Grama grass and sagebrush.
(8) Grama grass and chamiso.
(10) Grama grass and matchweed.
(23) Niggerwool.
(34) Galleta grass.
(37) Tussock grass.
(49) Sagebrush.
(50) Small sagebrush.
(52) Hop-sage.
(54) Shadscale.
(55) Salt sage.
(58) Winter-fat.
(59) White sage.
(61) Greasewood.
(62) Greasewood-shadscale.
(63) Greasewood-salt sage.
(64) Greasewood-saltgrass.
(65) Saltgrass.
(67) Seepweed.
(68) Samphire.
(102) Conifer timber.

REGION 6

(34) Galleta.
(37) Tussock grass.
(38) Sacaton.
(49) Sagebrush.
(50) Small sage.
(52) Hop sage.
(54) Shadscale.
(58) Winter fat.
(61) Greasewood.
(62) Greasewood-shadscale.
(63) Greasewood-salt sage.
(64) Greasewood-saltgrass.
(65) Saltgrass.
(67) Seepweed.
(68) Samphire.
(101) Woodland timber.

REGION 7

(1) Grama grass.
(2) Grama grass and wheatgrass.
(3) Grama grass and niggerwool.
(4) Gramagrass, niggerwool, and June-grass.
(7) Grama grass and sagebrush.
(8) Grama grass and chamiso.
(10) Grama grass and matchweed.
(22) Wire needlegrass.
(25) Matchweed.
(33) Tobosa grass.
(34) Galleta grass.
(35) Crowfoot grama.
(37) Tussock grass.
(38) Sacaton.
(42) Arizona fescue.
(46) Wet meadow.
(49) Sagebrush.
(64) Greasewood-saltgrass.
(65) Saltgrass.
(90) Willow (Salix species).
(92) Mountain mahogany.
(95) Southwest mountain brush
(96) Oak brush.
(100) Aspen.
(101) Woodland timber.
(102) Conifer timber.

REGION 8

(1) Grama grass.
(22) Wire needlegrass.
(25) Matchweed.
(27) Black grama.
(31) Black grama and wire needle-grass.
(32) Black grama and tobosa grass.
(33) Tobosa grass.
(34) Galleta grass.
(35) Crowfoot grama.
(37) Tussock grass.
(38) Sacaton.
(43) Short-lived grass type.
(61) Greasewood.
(65) Saltgrass.

REGION 8—Continued

(66) Pickleweed.
(69) Creosotebush.
(74) Creosotebush-desert grass.
(76) Creosotebush-ocotillo.
(78) Mesquite.
(79) Mesquite and chamiso.
(80) Mesquite and mesquite grass.
(85) Yucca desert grass.
(86) Black brush.
(87) Black brush grass.
(95) Southwest mountain brush.
(96) Oak brush.
(101) Woodland timber.
(102) Conifer timber.

REGION 9

(34) Galleta grass.
(37) Tussock grass.
(44) Giant wild-rye.
(49) Sagebrush.
(50) Small sagebrush.
(52) Hop-sage.
(54) Shadscale.
(55) Salt sage.
(56) Little rabbitbrush.
(58) Winter-fat.
(59) White sage.
(60) Coleogyne.
(61) Greasewood.
(62) Greasewood-shadscale.
(63) Greasewood-salt sage.
(64) Greasewood-saltgrass.
(65) Saltgrass.
(66) Pickleweed.
(67) Seepweed.
(68) Samphire.
(72) Shrub buckwheat.
(101) Woodland timber.

REGION 10

(39) Wheat bunchgrass.
(40) Curly bunchgrass.
(41) Mixed mountain grass.
(46) Wet meadow.
(47) Dry meadow.
(48) Mountain weed.
(49) Sagebrush.
(51) Scab sagebrush.
(53) Antelope-brush.
(90) Willows.
(91) Mountain brush.
(101) Woodland timber.
(102) Conifer timber.

REGION 11

(46) Wet meadow.
(47) Dry meadow.
(48) Mountain weed.
(49) Sagebrush.
(90) Willows.
(93) Chaparral.
(98) *Ceanothus cuneatus.*
(99) California mixed brush type.
(101) Woodland timber.
(102) Conifer timber.

REGION 12

(45) Weed grass.
(46) Wet meadow.
(47) Dry meadow.
(48) Mountain weed.
(90) Willows.
(94) Manzanita.
(98) *Ceanothus cuneatus.*
(99) California mixed brush type.
(102) Conifer timber.

REGION 13

(45) Weed grass.
(65) Saltgrass.
(71) Encelia and California sagebrush.
(90) Willows.

REGION 14

(45) Weed grass.
(46) Wet meadow.
(47) Dry meadow.
(94) Manzanita—brush type.
(97) Chamise.
(98) *Ceanothus cuneatus.*
(99) California mixed brush type.
(101) Woodland timber.
(102) Conifer timber.

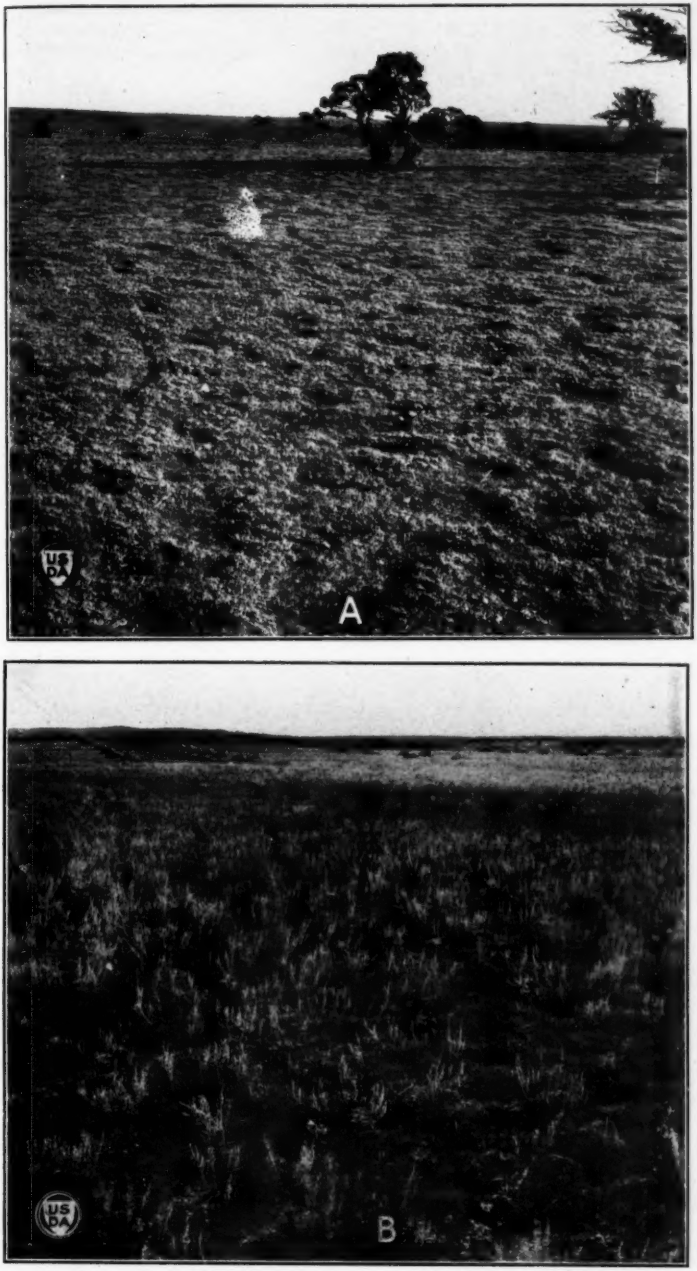
REGION 15

(36) *Hilaria rigida.*
(37) Tussock grass.
(60) Coleogyne.
(65) Saltgrass.
(66) Pickleweed.
(69) Creosotebush.
(70) Creosotebush and bur-sage.
(71) Encelia and California sage-brush.
(72) Shrub buckwheat.
(73) Desert brush type.
(75) Creosotebush and cacti.
(76) Creosotebush-ocotillo.
(77) Giant cactus and bur-sage.
(78) Mesquite.
(81) *Parosela arborescens* and *Lepidospartum squamatum.*
(82) Pluchea.
(83) *Atriplex lentiformis.*
(84) Desert sage.

PLATE 1[a]

A.–(1) Grama grass. East side Estancia Valley, N. Mex.
B.–(5) Grama grass and mountain sage. Havre, Mont.

[a] All photographs supplied by H. L. Shantz.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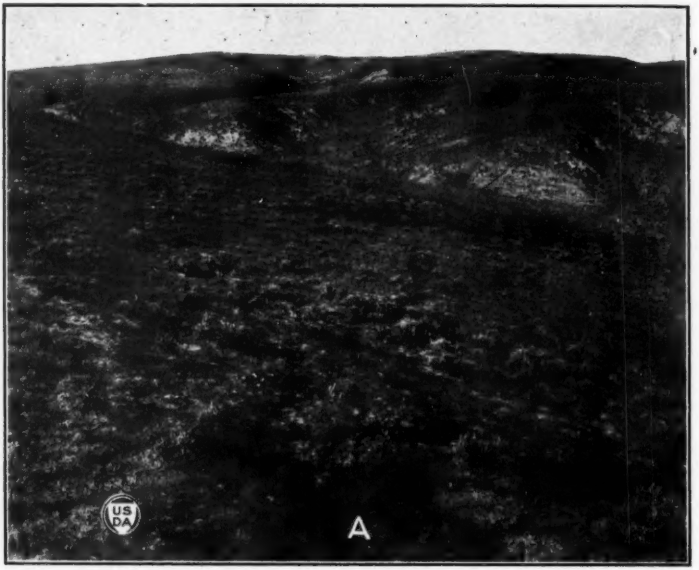
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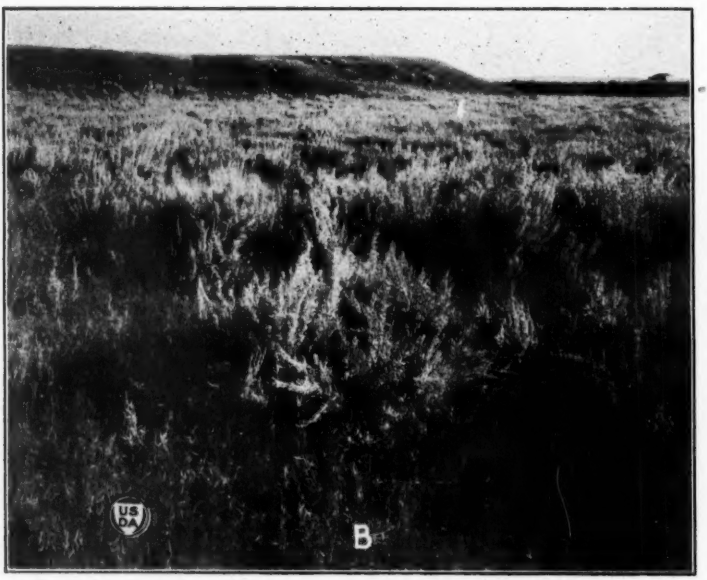
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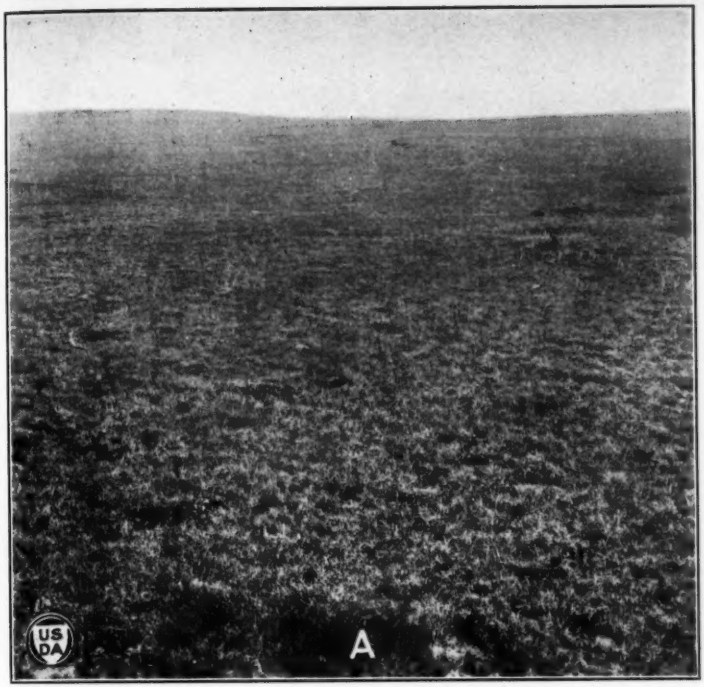
PLATE 2

A.–(6) Grama grass, niggerwool, and sagebrush. Storey, Wyo.
B.–(11) Grama grass and valley sage. Havre, Mont.

PLATE 3

A.–(13) Grama-buffalo grass. East of Limon, Colo.
B.–(15) Grama-buffalo and wire needlegrass. Yuma, Colo.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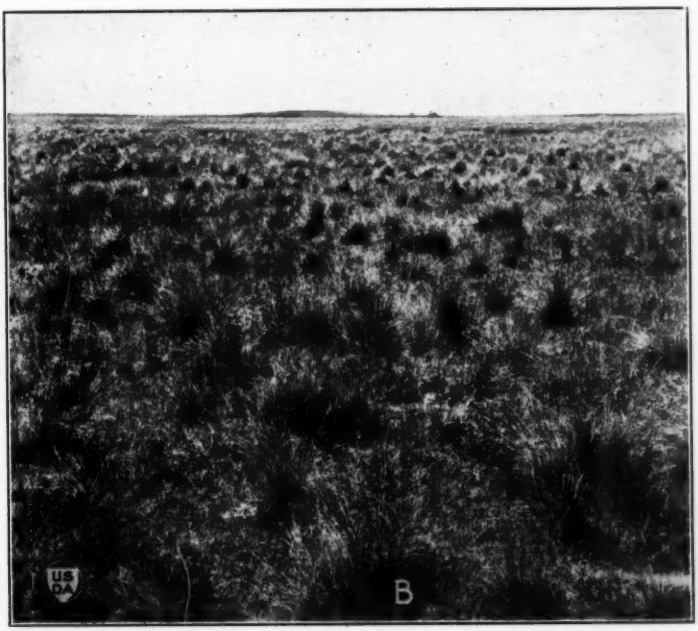
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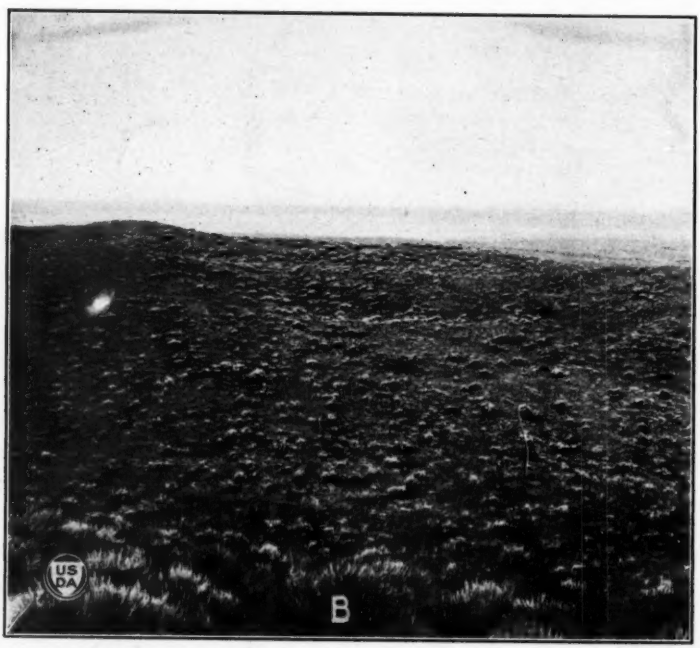

A.
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PLATE 4

A.–(20) Grama-buffalo grass and Gutierrezia. Southeast of Hartley, Tex.
B.–(30) Sand hills mixed. Wray, Colo.

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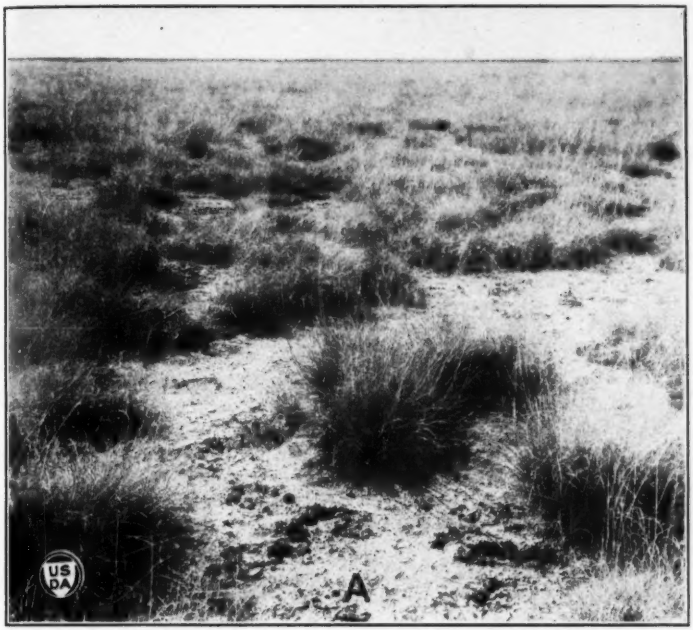
PLATE 5

A.–(37) Tussock grass. 15.2 miles south of Roswell, N. Mex.
B.–(39) Wheat bunchgrass. Heppner, Oreg.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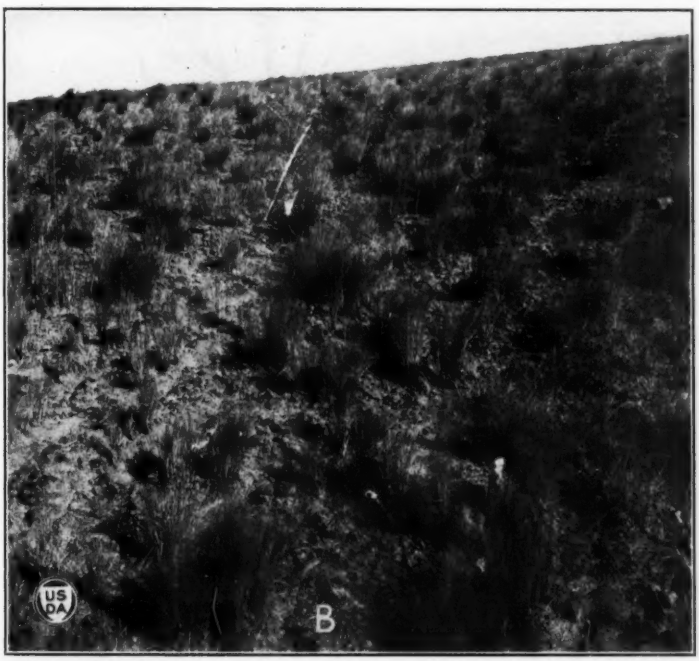
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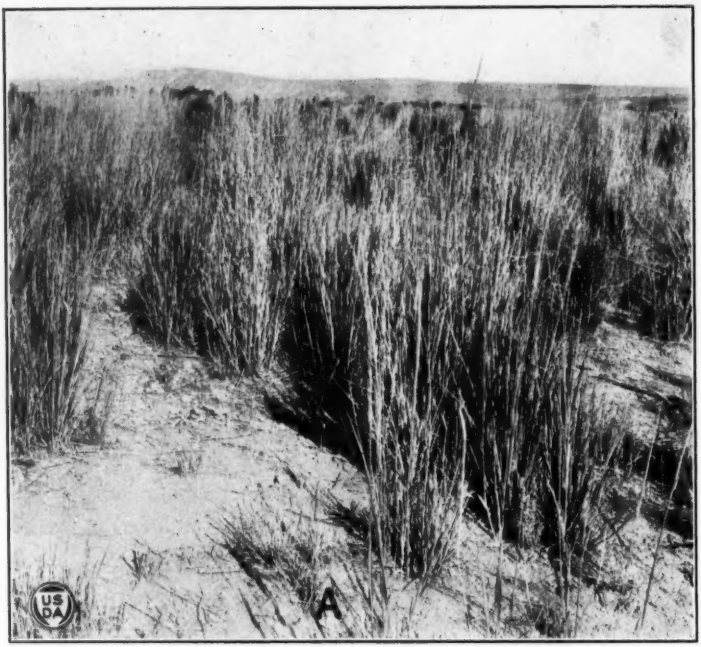

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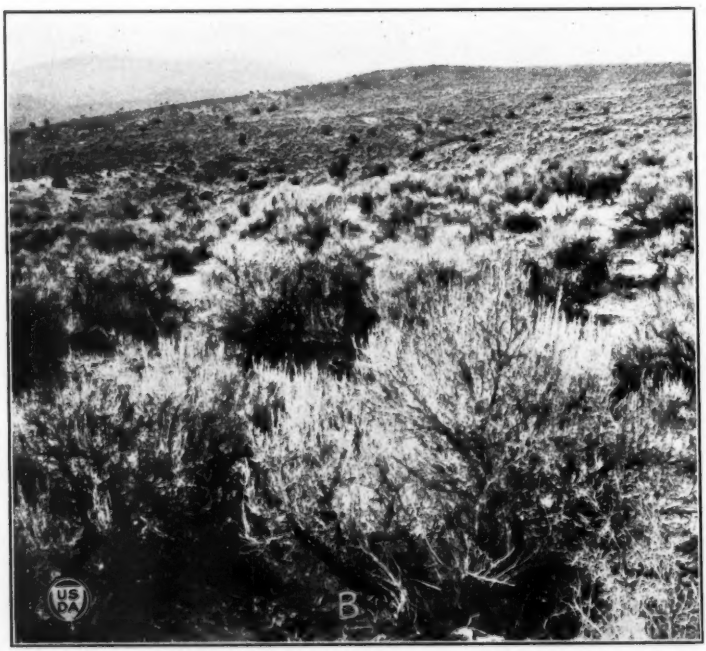

B.–

PLATE 6

A.–(44) Giant wild-rye. Harper, Oreg.
B.–(49) Sagebrush. Elko, Nev.

PLATE 7

A.–(50) Small sagebrush Burns, Oreg.
B.–(53) Antelope-brush. Millikin Valley, Oreg.

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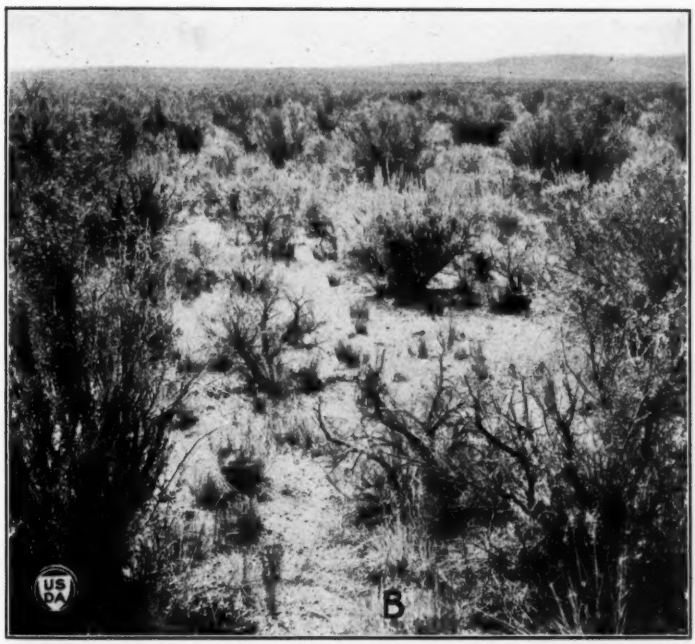
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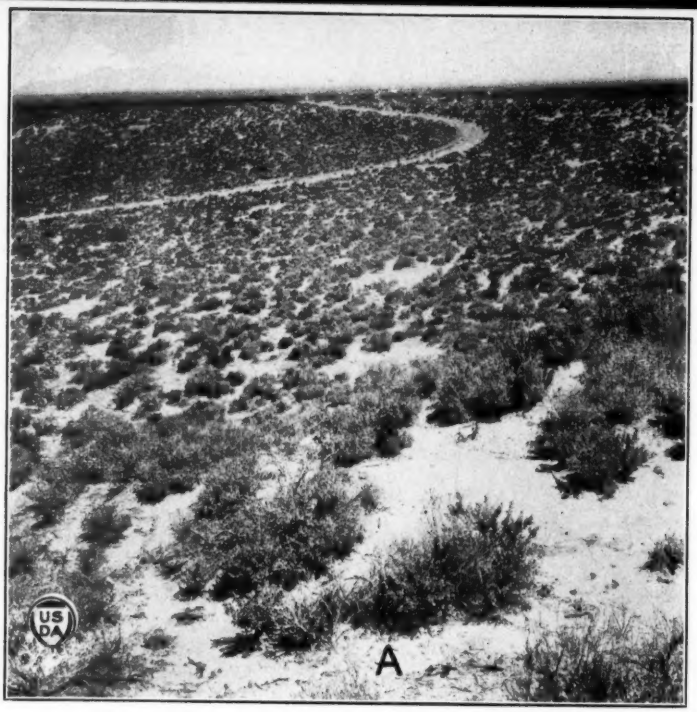
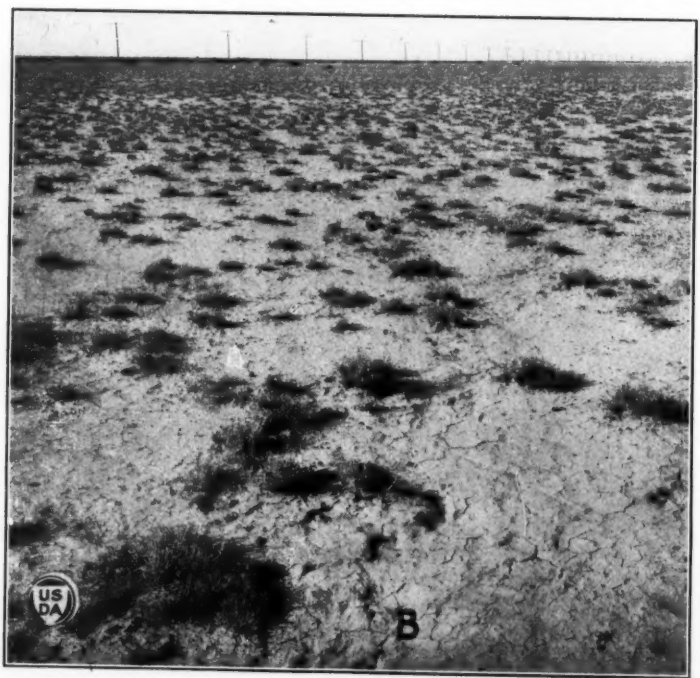




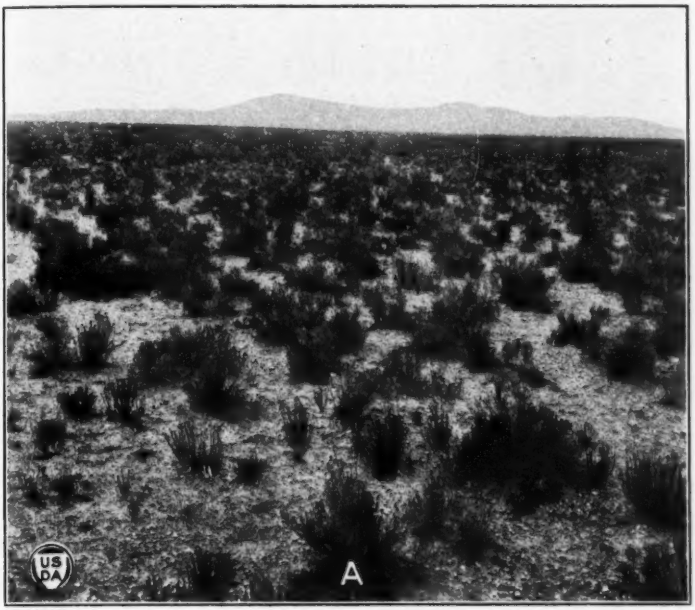
A.
B.

PLATE 8

A.–(54) Shadscale. Hollow beyond Grantsville, Utah.
B.–(55) Salt sage. Lund, Utah.

PLATE 9

A.–(56) Little rabbitbrush. Modena, Utah.
B.–(58) Winter-fat. Near Modena, Utah.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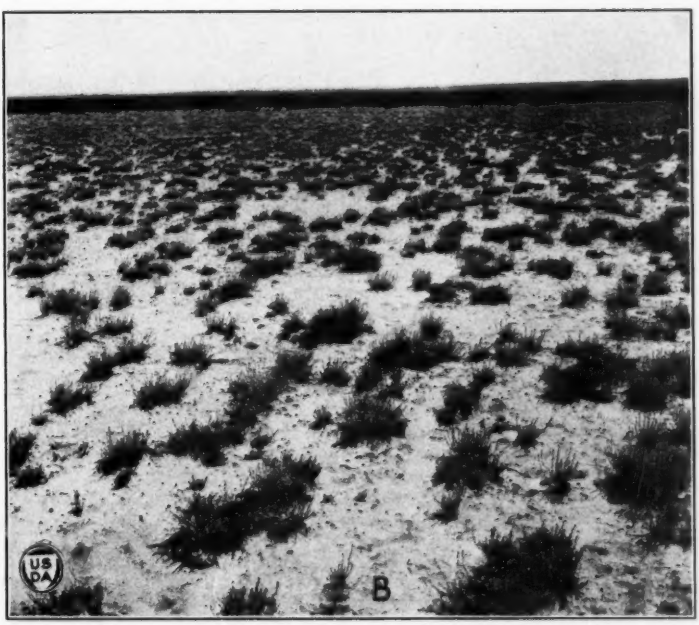
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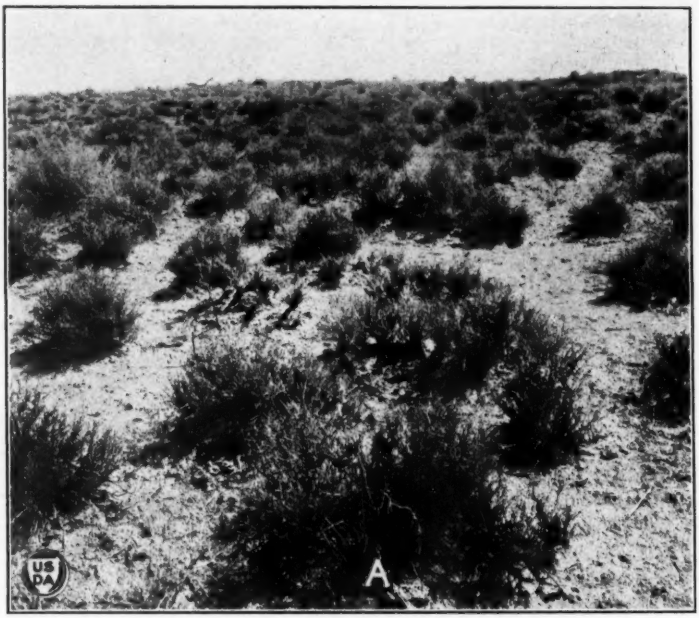

A

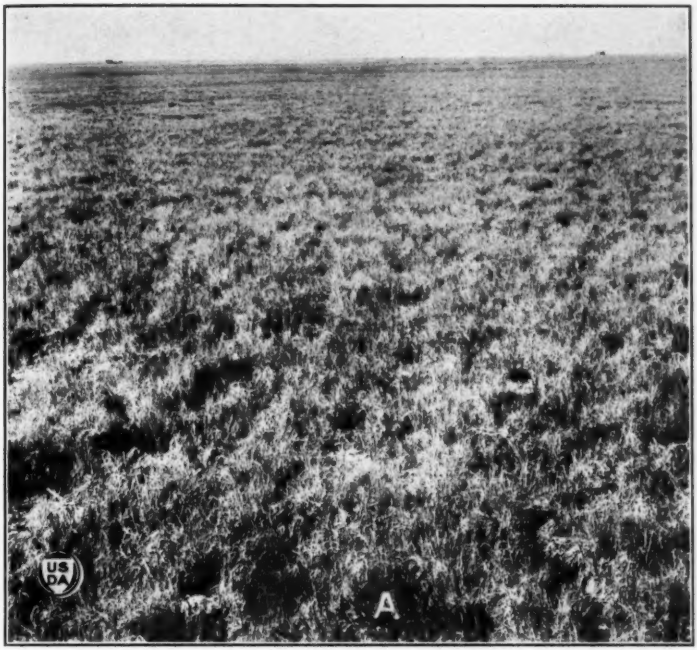
B

PLATE 10

A.–(60) Coleogyne. Near Bluff, Utah.
B.–(61) Greasewood. San Luis Valley, Colo.

PLATE 11

A.–(65) Saltgrass. Seven miles north of Willard, N. Mex.
B.–(66) Pickleweed. Lund, Utah.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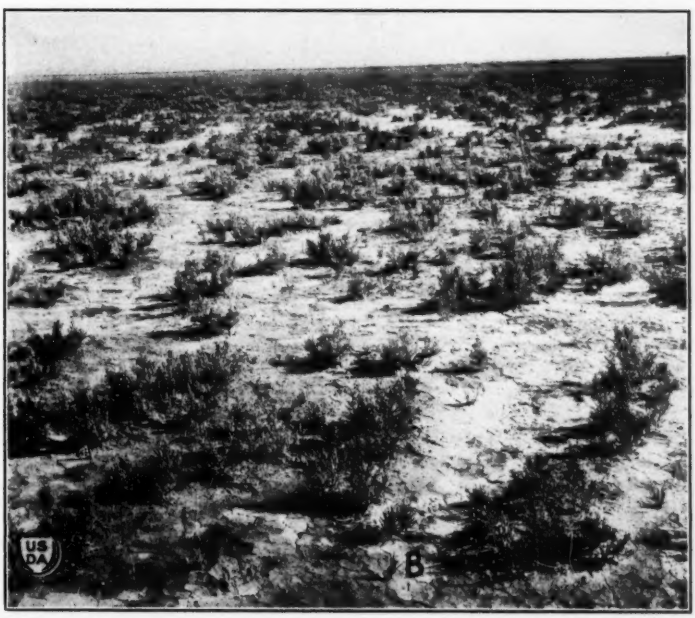
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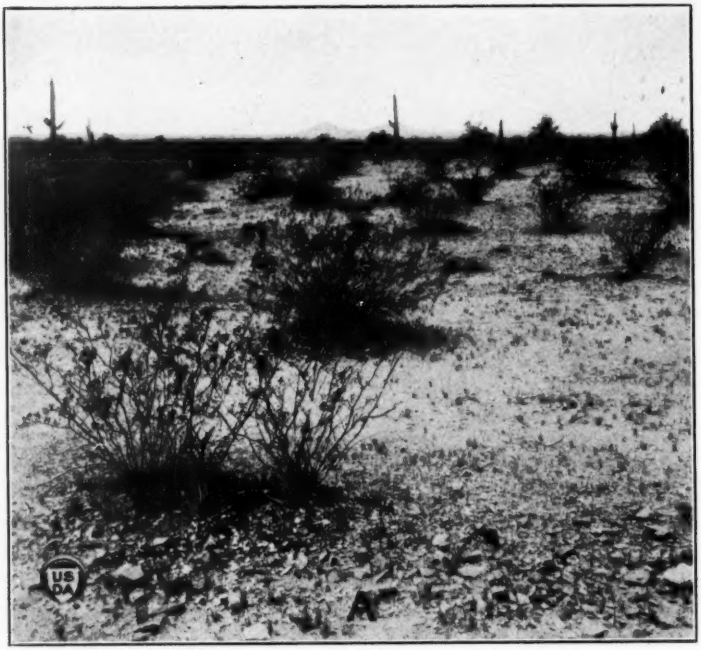
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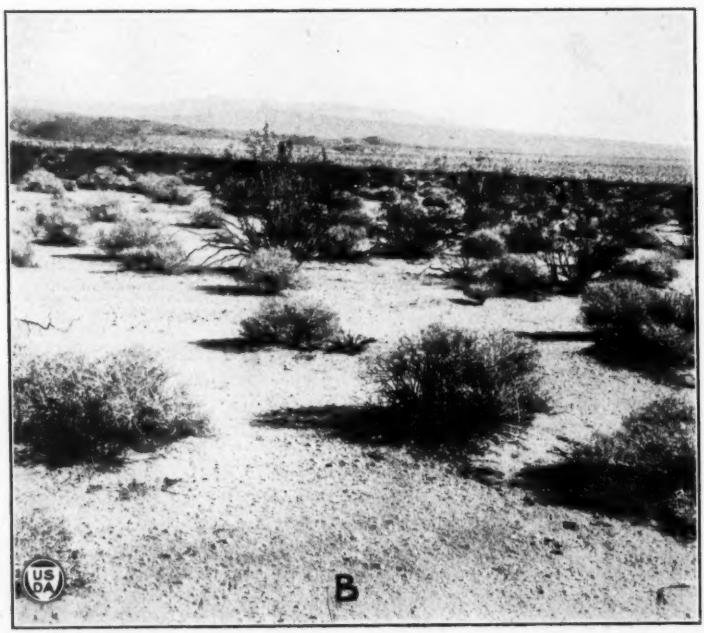
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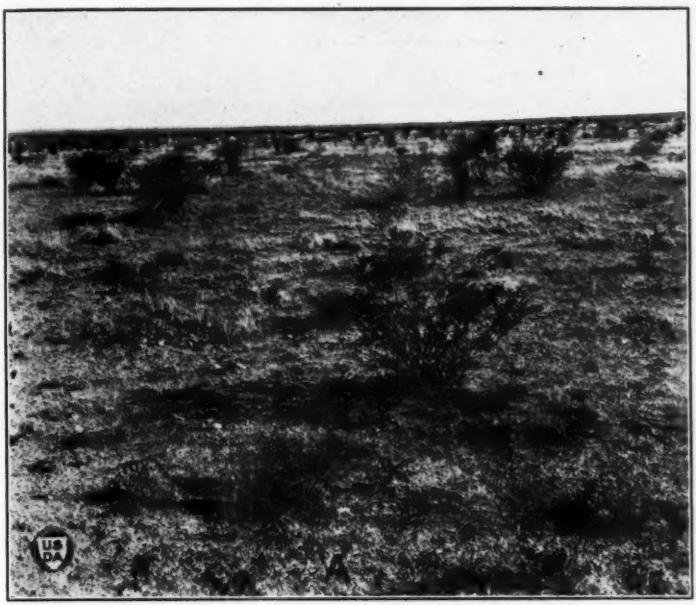
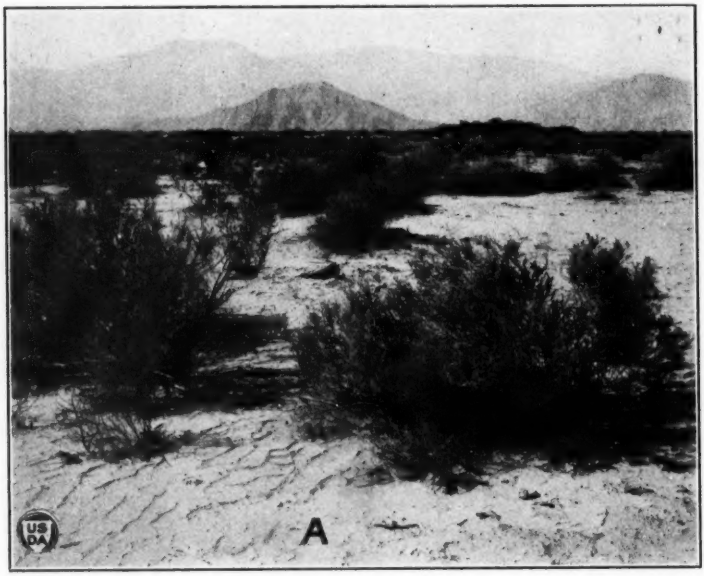
PLATE 12

A.–(69) Creosotebush. Sacaton, Ariz.
B.–(70) Creosotebush and bur-sage. Twenty-five miles east of Barstow, Calif.

PLATE 13

A.–(74) Creosotebush-desert grass. Six miles northeast of Hope, N. Mex.
B.–(78) Mesquite. Santa Rita Range Reserve, Ariz.

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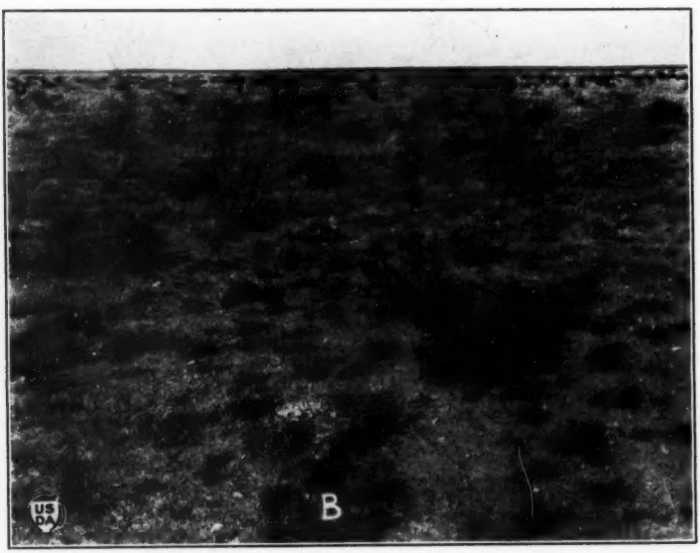
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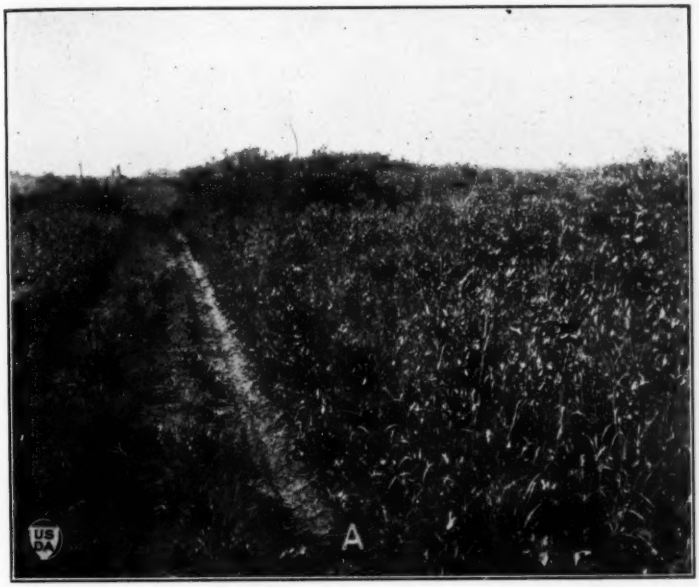
PLATE 14

A.–(84) Desert-sage. Thermal, Calif.
B.–(87) Black brush-grass land. West of Lakewood, N. Mex.

PLATE 15

A.–(89) Shinnery. Cheyenne, Okla.
B.–(91) Mountain brush land. Disappointment Canyon, Colo.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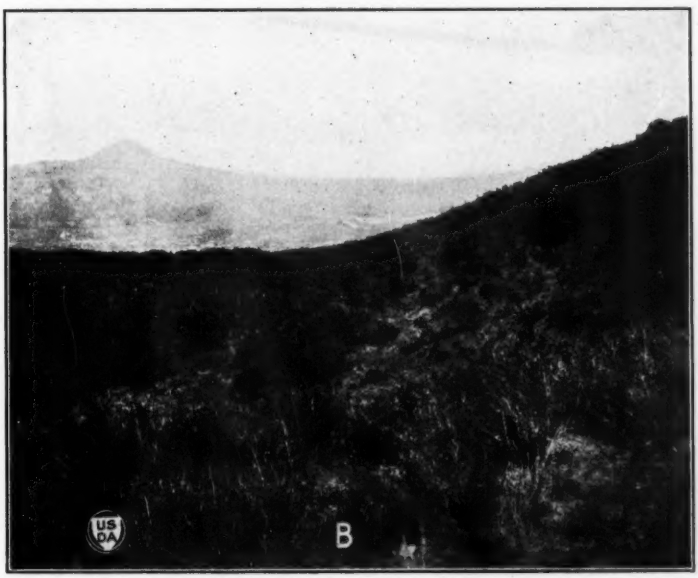
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PLATE 16

A.–(97) Chamise. San Diego, Calif.
B.–(98) *Ceanothus cuneatus*. Banning, Calif.

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THE DEVELOPMENT OF AMERICAN FOULBROOD IN RELATION TO THE METABOLISM OF ITS CAUSATIVE ORGANISM

By A. P. STURTEVANT,[1]

Agricultural Assistant, Bee Culture Investigations, Bureau of Entomology, United States Department of Agriculture

INTRODUCTION

American foulbrood is one of the two serious diseases affecting the brood of the honeybee. The specific cause of this disease is a pathogenic, spore-forming microorganism, known as *Bacillus larvae*. The occurrence of this organism in uniformly pure culture, accompanied by the gross effects of its activity, as manifested by the characteristic appearance and age of the diseased and dead larvæ, differentiates American foulbrood from the other serious brood disease of bees, European foulbrood. The latter disease is caused by an entirely different non-spore-forming organism, *Bacillus pluton*, which causes a different manifestation of gross symptoms, complicated by the action of various secondary invaders.

Certain limited facts concerning the characteristics of the various types of bacteria concerned in causing or associated with these brood diseases have been studied, from which various practical applications have been derived. As has been stated by Phillips (*39*)[2], "Bacteriological studies of bee diseases have been useful to practical beekeepers in explaining the reasons for success or failure with various treatments attempted. These studies have been especially important, however, because through them methods of laboratory diagnosis of the different diseases have been worked out."

Advancement in knowledge concerning the etiological and biochemical relationships of the brood diseases, particularly concerning differences in characteristics as related to gross symptoms, has been limited, however, because of the peculiar growth requirements of the causative organisms. There are fundamental differences between American foulbrood and European foulbrood, particularly as to characteristics of development, which, although recognized, have not been adequately explained by the incomplete data so far obtained on the metabolism of the causative organisms.

The present investigation was undertaken to obtain further data concerning the growth requirements of *Bacillus larvae*, the cause of American foulbrood, by which to explain these differences in the symptoms and development of the two diseases. Through improved methods of cultivation, a study has been made of factors concerned in the metabolism of *Bacillus larvae* correlated with certain hitherto unrecognized biochemical factors associated with the metabolism of the normal honeybee larva. The results obtained add materially to the knowledge of the biology of the brood diseases.

[1] Acknowledgments are due to Dr. E. R. Whitmore, professor of bacteriology and preventive medicine of the George Washington University, for much valuable advice and many suggestions, and to Dr. E. F. Phillips, apiculturist, Bureau of Entomology, United States Department of Agriculture, under whose direct supervision this work was done. Presented in part satisfaction of the requirements for the degree of doctor of philosophy at the George Washington University, April 21, 1923. This work was completed April 10, 1923.

[2] Reference is made by number (italic) to "Literature cited," p. 165-168.

THE RELATION OF CONTRIBUTING CAUSES TO THE COMPARATIVE DEVELOPMENT OF THE TWO SERIOUS BROOD DISEASES OF BEES

In order to understand the basis upon which the consideration of this problem has been developed, it is necessary to make a comparative study of certain of the characteristics of the two brood diseases, American foulbrood and European foulbrood, aside from their etiology. It will be apparent from this study that certain contributing causes, although recognized and described, have not been further analyzed to any extent, particularly in relation to specific etiology. The experimental work of the present investigation is concerned primarily with American foulbrood, however, since the causative organism, *Bacillus larvae*, can be isolated and grown in pure culture, while as yet no artificial medium suitable for the growth of *Bacillus pluton*, the cause of European foulbrood, has been devised.

RACE

It is an accepted fact that in American foulbrood the race or strain of bees has little or no relation to the development of or the resistance to the disease. This, aside from apparent lack of immunity or resistance of any of the races, may be explained partially by the fact that the decomposed material resulting from the death of the larvæ is of such a nature that the bees can not to any extent remove it from the combs after the disease has once become established. The dried-down masses (scales) are practically glued to the cell walls. *Bacillus larvae* forms resistant spores which allow the disease to be carried and spread almost indefinitely by means of the honey and old scales.

In European foulbrood, on the contrary, Italian bees seem to have some characteristic which makes them more resistant or vigorous in combating infection under the proper conditions. The results of bacterial decomposition of the diseased remains, even at their worst, are such that, if the colony is able to build up or is made sufficiently strong in worker bees, they are able to remove these remains, thereby removing the infection sufficiently to prevent its further development. *Bacillus pluton* does not form spores and lives only a comparatively short time under unfavorable conditions for growth, as in honey or on long drying. Furthermore, as has been demonstrated by the writer in a previous paper (*45*), this apparent resistance of the Italian bees was observed to be due largely to the racial characteristic of removing all foreign materials more promptly from the hive than do common black bees or hybrids, rather than to any natural resistance or immunity to the disease.

STRENGTH OF COLONY

If a colony of bees has been exposed to infection from American foulbrood, the strength of the colony apparently has no direct relation to the development of the disease, except that strong colonies are usually the ones which rob the weaker infected colonies, thereby spreading the infection through the apiary. As suggested above, European foulbrood attacks primarily the weak colonies which have an insufficient force of bees to remove the infected material. Diseased combs from such a colony can be placed in a strong healthy colony of Italian bees with no resulting disease. This would be fatal in the case of American foulbrood.

SEX

There has been slight mention in the literature of the relation of the sex of the bee larvæ to the development of disease. Phillips (*38*) states with regard to European foulbrood: "A symptom of greatest importance is the fact that the

disease attacks drone and queen larvæ nearly as quickly as those of the workers. The tendency of this disease to attack queen larvæ is a serious drawback in treatment. Frequently bees of a diseased colony attempt to supersede their queen but the larvæ in the queen cells often die, leaving the colony hopelessly queenless. The colony is thus depleted rapidly."

In American foulbrood, according to Phillips (*39*), "Usually the disease attacks only worker brood, but rare cases are found in which queen and drone brood are diseased." White (*55*) states, however: "That worker, drone, and queen larvæ are all susceptible to the disease has been demonstrated during these [White's] studies. Affected drone brood is encountered less often in the diagnosis of this disease than in that of European foulbrood. The writer has encountered queen larvæ affected by American foulbrood in experimental colonies only, although very probably diseased queen larvæ do occur in nature also." A few samples of diseased brood containing American foulbrood sent to the Bee Culture Laboratory for diagnosis have been found to contain affected drone larvæ as well as one or two cases of diseased queen larvæ. Although beekeepers believe that in American foulbrood drone brood is so seldom affected that the absence of diseased drone brood is a diagnostic character, the fact that occasionally drone larvæ do die of the disease makes it possible that some other factor than nonsusceptibility of sex is concerned. No accurate data are available on this subject. The work of this paper is concerned only with worker brood, because the great preponderance of worker brood affected gives slight importance to the comparatively few drone larvæ in the average colony.

AGE

The general characteristic difference in age between larvæ dying of American foulbrood and those dying of European foulbrood, mentioned at the beginning of this paper, has been one of the chief factors in the differentiation between the two diseases. Originally there was considered to be only one disease, "foulbrood." Although beekeepers have long known that brood of various ages is attacked by brood disease, it seems not to have been until about 1880 that the difference in age at the time of attack was used to separate foulbrood into two distinct forms, one "easily curable" and the other "virulent." Dzierzon (*21*) was the first thus to differentiate definitely into two types of disease, according to the difference in symptoms and age at time of attack. He stated that in the curable disease, "More of the larvæ die still unsealed, while they are still coiled in the bottom of the cell * * *. The brood which does not die before sealing mostly attains to perfection * * *. This is exactly the reverse in the malignant kind of foulbrood. In this the larvæ do not generally die before they have raised themselves from the bottom of the cell, have been sealed and begun to change into nymphs."

Cheshire (*13*) who probably was the first to investigate the bacteria associated with what, in the light of present knowledge, is known as European foulbrood, was inclined to agree at first with the distinctions made by Dzierzon. He soon stated (*14*), however, that Dzierzon was in error and that there is only the one disease, foulbrood, which he supposed was caused by an organism to which he gave the name *Bacillus alvei*. Cheshire and Cheyne (*15*) described *Bacillus alvei* as a spore-forming bacillus which they constantly found associated with a diseased condition of the brood and recognized only as "foulbrood." The results of this work caused considerable confusion to beekeepers and investigators, both in this country and abroad, for more than a decade.

In this country some time after 1890 it became evident to certain beekeepers, particularly in New York State, that they were dealing with two distinct diseases. The newly recognized form, which was found to attack the coiled larvæ,

was at first erroneously called "black brood," to distinguish it from the "foulbrood" of sealed larvæ. "Black brood" assumed epidemic proportions in New York State by 1897. This gave rise in American beekeeping literature to descriptions of two distinct diseases, as far as the age of the larvæ attacked and the appearance from the resulting decomposition were concerned.

RESULTING DETERMINATION OF ETIOLOGY

As a result of the increasing devastation by this new disease, work was started in New York State in 1902 (*33*), which was later carried on by White (*49*, *50*), on the bacteriology of these brood diseases, by which doubt was cast upon *Bacillus alvei* being the cause of any disease, although it was found to be associated only with European foulbrood. Furthermore, a new spore-forming bacillus distinct from *Bacillus alvei* was observed and cultivated on special culture media from the disease attacking the sealed larvæ. This organism was at first designated *Bacillus* X but was later named *Bacillus larvae* (figs. 1 and 2). Subsequently this was found to be the cause of American foulbrood by experimental inoculation of healthy colonies with pure cultures (*51*). The symptoms

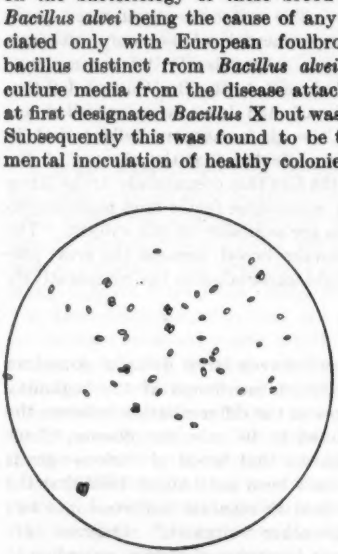

FIG. 1.—Spores of *Bacillus larvae*. (McCray (*51*))

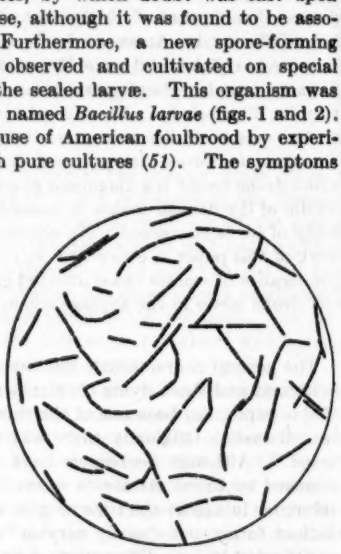

FIG. 2.—Vegetative rod form of *Bacillus larvae*. (White (*55*))

were accurately described and differentiated by Phillips (*37*), definite new names being used for the first time in order to eliminate confusion, as follows: American foulbrood, formerly known as "foulbrood" ("Usually the larvæ are attacked at about the time of capping, and most of the cells containing infected larvæ are capped"); and European foulbrood, originally called "black brood" ("This disease attacks the larvæ earlier than does American foulbrood, and a comparatively small percentage of diseased brood is ever capped").

Maassen (*27*) in Germany described at about the same time what is now accepted as the same organism as *Bacillus larvae*, a spore-forming organism constantly found to be present in the diseased brood dying after sealing, "Nymphenseuche." He gave the name *Bacillus brandenburgiensis* to this organism. Burri (*12*) in Switzerland also recognized the fact that the spores present in large numbers in scales in the "nymph" disease were a new species that was difficult of cultivation.

White (*52*) later showed conclusively that *Bacillus alvei* is not the cause of European foulbrood but is only one of several secondary invaders. He demonstrated that the probable cause of European foulbrood is a nonspore-forming organism which he called *Bacillus pluton*. This organism develops before the

death of the larva in the intestinal tract and usually kills before sealing takes place, as differentiated from American foulbrood as described above. Unfortunately, as yet it has been impossible to grow this organism in pure culture on artificial culture media.

Further work has been done by various investigators on certain laboratory phases of the bacteriology and diagnosis of the two diseases, but no additional information has been obtained concerning the etiological and biochemical relationships of the causative organisms which would aid in the solution of the present problem.

BASIS FOR INVESTIGATIONS

Throughout all the discussion of symptoms of the brood diseases in the literature, particularly in relation to the different ages at which the diseases attack during the life history of the larvæ, there has been no adequate explanation of the reason for this apparent fundamental difference.

Maassen (*28*) in the case of American foulbrood made the observation that, "according to the microscopic findings from section preparations, *Bacillus brandenburgiensis* [*Bacillus larvae*] does not come to luxuriant development in the intestine of the larva, though this is the case with *Bacillus alvei* and with *Streptococcus apis* [in 'sourbrood']. It finds much more promising nourishment in the fat bodies of the larva. Apparently the bacillus finds opportunity to press its way into the fat bodies shortly before the pupation of the bee, at the beginning of the natural changes in the intestinal tube. From this it seems clear why the larvæ containing *Bacillus brandenburgiensis* die after sealing." In part this is probably correct, since it may easily be observed that soon after capping the tissues of the healthy larva become more or less granular and watery in consistency, at which time it is almost impossible to distinguish the intestinal tract. It is also difficult to remove the larva in this condition from the cell without rupturing the skin envelope. This process is described more in detail later. It does not explain, however, why the spores of *Bacillus larvae* do not germinate and increase in numbers sufficiently to kill the larva much earlier during the feeding period, as in the case of European foulbrood. A vague and only partially correct suggestion was given in an earlier paper by the writer (*46*), in which the following theory was stated: "*Bacillus larvae* gains entrance to the larva generally in the spore stage, in the larval food. This occurs at about the same stage as in European foulbrood, while the larva is still coiled in the cell. Only rarely, however, do coiled larvæ die. This is apparently because it takes some time for the resting stage spores to germinate into the active vegetative rods. This causes death, as a rule, to occur later in the life history of the larva."

RELATION OF THE BROOD DISEASES TO THE LIFE HISTORY OF THE HONEYBEE LARVA

The development of the honeybee may be divided in general as follows: After the egg is laid there is a period of three day's incubation before it hatches into the larva. The larval stage, during which active feeding and growth occur, comprises four and a half to five and a half or six days. At the end of the feeding period the larva is sealed in the cell, where it spins its cocoon. Metamorphosis then occurs, and the fully formed adult bee emerges in about 12 days, making a complete developmental period of approximately 21 days. According to White (*53*), there is a prepupal period in healthy brood of four days after sealing occurs before the actual change in the external form to that of the adult bee takes place. During the first two days after capping, the larva is active in the cell, consuming any remaining food and spinning a cocoon. Some time during this period according to Straus (*43*), or just previous to capping according to Zander (*57*),

the larval intestine, which up to this time has been a blind sac, is connected with the end gut, allowing defecation to take place. There is then two days of quiescence, during which the larva extends in the cell and lies motionless, while internal changes preparatory to metamorphosis occur (figs. 3 and 4). These changes (*7*) consist of the almost complete histolysis of the fat body of the larva in order to furnish nutriment for the formation of imaginal tissues. This is made possible by the physiological and morphological changes occurring in this stage of the development of the larva. Extended investigations have been made of these physiological and morphological changes, but they need not be summarized further here, since the present work has been solely of a biochemical character. It is noticeable, however, that the intestines of mature larvæ even for a short time after capping are full of material colored by the pollen content, while the intestines of the prepupæ, after they have extended in the cell, are colorless.

FIG. 3.—Healthy prepupa approximately 8 days old, having reached the quiescent stage. This is the age at which the majority of larvæ die from American foulbrood. End view. (White (*55*))

It is during the latter two-day prepupal period that according to Maassen (*28*) the invasion of the fat body by *Bacillus larvae* occurs and that according to White (*55*) the majority of the brood dies in American foulbrood.

In European foulbrood, on the contrary, the majority of the larvæ in typical cases of this disease die before sealing and after reaching an age of 3½ to 4 days from the time of hatching of the egg (*56*) (fig. 5). In certain abnormal cases in European foulbrood death may occur after capping (*46*), but this almost always occurs during the first two days of the prepupal stage, when the larva in most cases is still moving about in the cell, usually causing a gross appearance quite different from that of brood dead of American foulbrood.

PRELIMINARY EXPERIMENTS

While studying the bacterial flora associated with the early stages of European foulbrood in the larval intestine certain results were obtained which suggested a possible explanation of the delayed development in American foulbrood. Until death takes place in European foulbrood the growth of the organism causing the disease and certain secondary associated forms occur only within the intestine (*52*); that is, within the peritrophic membrane, but not in actual contact with living tissues of the larva. It is only after death that the secondary invaders, particularly *Bacillus alvei*, invade the body tissues (*45*).

FIG. 4.—Healthy prepupa Side view. (White (*55*))

Another important distinction which must be considered is that the feeding of the larva is not the same throughout larval life. Von Planta (*40*) has shown that for the first part of the feeding period one type of food is used by the larva and that at a later stage a food different in chemical and physical composition is provided. Young larvæ receive a food for a time after hatching that is much richer in fat and albuminous material but lower in sugar content than that fed to older larvæ. The food of the older larvæ, which is known to consist mainly of honey or nectar and pollen, is much higher in sugar content, while there is a considerable decrease in fat and albuminous material. The sugar in the food of the older larvæ, particularly

that of larvæ at the age when European foulbrood makes its attack, comprises nearly 45 per cent of the dried substance, or nearly 14 per cent of the fresh substance. From these facts it may be assumed that, because of the great amount of food given the larva at this age, there must be present, in the larval intestine, at all times during the active feeding period, considerable amounts of this food rich in sugar unassimilated, up to and even after active feeding ceases. A number of larval and prepupal intestines were dissected from healthy larvæ and tested roughly with Benedict's qualitative solution (*34*) for the presence of reducing sugars. The results indicated the presence of relatively large amounts of reducing sugar in the intestines of larvæ just prior to sealing. Little or no reducing sugar could be demonstrated in the intestines of sealed larvæ or prepupæ.

It may therefore be assumed that certain of the organisms associated with the early stages of European foulbrood are able to grow in the presence of a high sugar concentration. Experiments were devised in which a medium containing 10 per cent dextrose was used. It was found that while a few types of organisms, such as one resembling *Streptococcus apis* (*28*), could be grown in varying numbers, an organism similar to that described by Maassen (*29*), resembling the larger forms of *Bacillus pluton*, called *Bacillus lanceolatus*, could be isolated and grown from over 50 per cent of the samples cultured. As described by the writer (*47*), "This organism was found to grow best on a 10 per cent dextrose yeast extract agar with a reaction slightly acid. It is differentiated from *Bacillus pluton* and *Streptococcus apis* in being gram-negative, and does not grow readily if at all in media without sugar." From these studies it was suggested that possibly this comparatively high sugar content of the unassimilated food in the larval intestine may have an influence on the germination of the spores and growth of *Bacillus larvae* and that a change may occur when the sugar content is sufficiently reduced by assimilation in the larval intestine after it has been capped and when the intestines have been emptied by the opening of the ventriculus into the end gut. Therefore, with these preliminary observations as a basis, experimental work on this subject was begun during the spring of 1922.

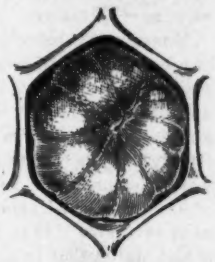

FIG. 5.—Healthy coiled larva at age of maximum intestinal sugar content and approximately the age when the majority die from European foulbrood. (White (*56*))

GROWTH OF BACILLUS LARVAE IN CULTURES IN RELATION TO VARIATION IN SUGAR CONCENTRATION

The first step in the substantiation of this theoretical assumption is to determine whether there is a correlation between germination of the spores of *Bacillus larvae* and vigor of vegetative growth and variations in concentration of reducing sugars in culture media. Ordinary culture media are unsuitable for the growth and isolation of *Bacillus larvae;* in fact, one of the confirmatory tests for this organism in laboratory diagnosis of American foulbrood (*31*) is the absence of growth on plain beef infusion agar plates, since the spores will not germinate thereon. There are rarely any secondary invaders associated with *Bacillus larvae* in the decayed material, and these plates practically never show growth.

GROWTH REQUIREMENTS OF BACILLUS LARVAE

Various special culture media have been devised which answer more or less satisfactorily the requirements for the ordinary growth of the organism. The

spores of *Bacillus larvae* will germinate and grow feebly on an agar medium in the preparation of which healthy bee larvæ are used as is meat in ordinary culture media, sterilizing as usual by heat in an autoclave (*49*). However (*51*), if a broth made by macerating healthy bee larvæ in several times their volume of water is sterilized without heating by filtering through sterile bacteria-proof filters and then is pipetted aseptically into tubes of previously sterilized liquefied agar cooled to 50° C., the resulting medium gives much better growth. This medium is nevertheless unsatisfactory, owing to difficulties of preparation, and particularly because of lack of material for its preparation except during the brood-rearing season. White (*54*) therefore devised a medium which consists of a suspension of the yolk of an egg aseptically in 70 cc. of sterile water, 1 cc. of which suspension is added by sterile pipette to each 5 cc. of ordinary sterilized tubed agar medium which has been melted and cooled to 50° C. Growth occurs on this medium quite abundantly, although with the technic described great care must be taken to prevent contamination.

Maassen (*28*) has also devised a medium made from a mixture of equal parts of a broth from calf or pig brain and a solution of egg albumin in water, to which 1.8 per cent agar and 1 per cent each of Witte's and Chapoteaut's peptone are added, after which it is filtered, tubed, and sterilized. This medium gives an almost neutral or weakly acid reaction to blue litmus paper. Maassen also found that the vegetative forms develop abundantly if grown on a meat and water medium if it is acid in reaction and if 0.25 per cent of pollen and 1.5 per cent of Aschmann's or Chapoteaut's peptone are added, but that the former medium is more favorable. Both media are found to deteriorate on too much heating. It is also stated that in acid peptone bouillon, in bouillon of bee larvæ, and in the brain bouillon, the bacillus may be cultivated, although growth is slow, the bouillon becoming weakly turbid and a thick slimy deposit gradually being formed.

For the purpose of the present experiments, after consideration of the advantages or disadvantages of the various media so far described, a modification of the egg-yolk suspension medium of White was adopted as the most satisfactory general medium. During the course of the experiments some modifications were made both in the medium and in the technic of preparation.

PREPARATION OF YEAST-EXTRACT AGAR BASE

Because of most satisfactory results in other work with various brood disease cultures, a yeast-extract agar described by Ayers and Rupp (*2*) was used instead of beef infusion agar as a base, because of the ease of preparation and the uniformity of the medium. Spores of *Bacillus larvae* on the surface of a slant of this agar germinate to some extent on this medium alone, and vegetative cultures from egg-yolk suspension agar transferred to the yeast medium grow fairly vigorously. The addition of egg-yolk suspension to the yeast-extract agar increased the vigor of growth and longevity of cultures.

One liter of the yeast extract agar is prepared as follows:

Dried yeast	grams	10
Peptone	do	10
Buffer (sodium glycero-phosphate)	do	5
Water	cc	500

This is heated in flowing steam for one-half hour, then adjusted to a hydrogen-ion concentration of $P_H = 7.6$ to 7.8 by the colorimetric method of Clark and Lubs (*16, 17*). The broth is then boiled for one minute over an open flame and filtered through filter paper on a perforated porcelain funnel, using siliceous earth to clarify. To this broth is added an equal amount (500 cc.) of double strength

(3 per cent) solution of agar, washed and filtered by the method described by Ayers, Mudge, and Rupp (*3*). The final hydrogen-ion concentration reaction is adjusted so that upon addition of 1 cc. of the egg-yolk suspension to 10 cc. of the yeast-extract agar the reaction is about $P_H = 6.8$. The normal hydrogen-ion concentration value of the contents of the larval intestine at various ages during the active feeding period with honey and pollen and just after sealing averages $P_H = 6.8$, varying to slightly more acid with the amount and type of pollen in the food material. Intestines were dissected out from the larvæ and macerated in 10 cc. of neutral distilled water and compared colorimetrically with known buffer solutions, using brom thymol blue as an indicator. Fabian and Parks (*22*) found this value to be $P_H = 6.6$ by macerating the entire larva in water. From earlier unpublished work by the writer, as well as by the above-mentioned investigators, the optimum hydrogen-ion concentration for the growth of *Bacillus larvæ* was found to be approximately $P_H = 6.8$. The yeast extract medium is tubed, sterilized in the autoclave at 15 pounds pressure for 15 minutes, and stored until needed.

PREPARATION OF EGG-YOLK SUSPENSION

The egg yolk can be diluted much more than was directed in the original formula with even better results, the more dilute suspension giving a more transparent medium with fully as profuse growth. A wide-mouthed flask containing 200 cc. water, sterilized with a cotton plug protected by a paper cap, is used for each egg yolk. At times, from 0.5 per cent to 1 per cent of a neutral buffer salt is added to the water previous to sterilization. This holds in check the slow increase in acidity observed on long standing. A small amount of normal sodium hydroxid (2 to 3 cc.) is also added to the flasks before sterilization to bring the resulting reaction of the egg suspension nearer to the desired reaction for the final medium.

APPARATUS TO REPLACE PIPETTING

An apparatus was devised (fig. 6) which to a great extent eliminates the danger of contamination of agar tubes when adding egg-yolk suspension, and also makes possible the preparation of a large quantity of medium in a short time. As a rule egg-yolk suspension can be stored or withdrawn at any time after the apparatus has been set up, until all used up, unless the egg yolk itself is not sterile. A two-holed rubber stopper, of correct size to fit the flask containing the egg-yolk suspension, is fitted with two tubes, one of small bore to reach nearly to the surface of the liquid when placed in the egg-yolk flask, and a second larger tube fitted flush to the inner surface of the stopper, protruding ouwartd about 1½ inches. A piece of rubber tubing 5 inches long is fitted to this tube, closed with a pinchcock. To this rubber tube is attached a delivery tube which passes through another rubber stopper placed in one end of a glass cylinder 1¼ inches in diameter and 4 inches long, to about half its length. This forms a protective bell for the delivery tube similar to that used in filling vaccine or antitoxin ampules. The

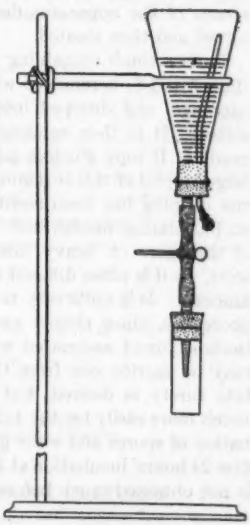

FIG 6.—Apparatus to replace pipetting of egg-yolk suspension

entire apparatus is sterilized in the autoclave, using a temporary empty flask into which the stopper for the culture flask is placed, and all is wrapped in paper with a paper protective cap over the open end of the delivery bell. Before use, the apparatus is removed from the paper and the stopper is carefully removed from the empty flask so as to prevent contamination and is fastened firmly in the flask containing the egg-yolk suspension. After placing the pinch cock in position, the apparatus is carefully inverted and hung on a ring stand. The small-bore glass tube in the flask now reaches a little above the surface of the liquid and serves for an air inlet. By means of this apparatus, sterile egg-yolk suspension can be added to tubes of sterile base medium, with little danger of external contamination, by inserting the tube under the protective bell.

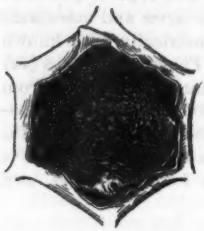

FIG. 7.—American foulbrood scale. End view. (White (55))

METHOD OF ISOLATION OF PURE CULTURES OF BACILLUS LARVAE

When medium is desired for the isolation or cultivation of *Bacillus larvae*, tubes of the yeast-extract agar are melted in a water bath and cooled to 55° C., after which from 1 to 2 cc. of egg-yolk suspension is added for each 10 cc. of base, by means of the apparatus described above. The contents of the tubes are well mixed and then slanted.

From a comb containing decaying material dead of the disease, a dried scale (figs. 7 and 8) is removed with a sterilized needle scalpel (also used for removing cappings) and dropped into the water of condensation in the culture tube to soften. It is then smeared over the surface of the agar with an inoculating needle. If ropy gluelike material is available it is more satisfactory (fig. 9). A large loopful of this is removed from the cell, from which the capping has been aseptically removed by means of an inoculating needle, and is streaked over the surface of the agar. A heavy initial inoculum gives best results, as it is often difficult to obtain growth with a small amount. It is quite easy to obtain pure cultures by this procedure, since almost never are secondary contaminations found associated with *Bacillus larvae*. Plating may be carried out from these initial cultures if absolute surety is desired, but initial growth is obtained much more easily by the tube culture method. Germination of spores and some growth take place during the first 24 hours' incubation at 37° C., but maximum growth is not obtained much before 48 hours.

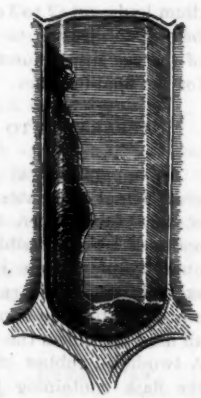

FIG. 8.—American foulbrood scale. Side view. (White (55))

EXPERIMENTAL PROCEDURE, USING AGAR SLANTS

To determine whether there is a correlation between germination of spores and vegetative growth of *Bacillus larvae* and the concentration of sugar in the culture medium, a series of tubes is prepared with varying percentages of dextrose, from 0.5 per cent to 10 per cent (Table I). These are prepared by adding the required amounts of dextrose to 50 cc. portions of the yeast-extract agar base, which is then tubed and sterilized at 10 pounds pressure for 15 minutes. On cooling to 55° C., 1 cc. of sterile egg-yolk suspension is

added to each tube and it is then slanted. Series of agar slants varying in sugar concentration are inoculated with either vegetative cultures or diseased material containing only spores. To determine spore germination an approximately uniform amount, about one 2-mm. loopful of ropy material, when available, is used for inoculation of slants, otherwise a scale softened as described above. If no visible growth takes place after 48 hours' incubation, stained smears are made, to determine whether any germination has occurred. In the case of the determination of growth from vegetative culture, a single uniform streak is made on the agar slant, using one 2-mm. loopful of growth from a 48-hour culture of *Bacillus larvae* prevously isolated and cultivated. After 48 hours' incubation, as well as after about one week, comparative observations are made of the relative amount and character of the growth. Where little or no growth has occurred, stained smears are made from the streak to see what has happened to the organisms. These experiments were carried out with a number of different strains of vegetative cultures and from a number of different samples of American foulbrood.

FIG. 9.—Partially decomposed American foulbrood larva at the stage of ropy consistency. (White (55))

TABLE I.—*The effect of varying the sugar concentration in egg-yolk suspension medium (1) on germination and vegetative growth from spores; and (2) on vegetative growth from vigorous vegetative cultures of Bacillus larvae.*[a]

Test material	Per cent dextrose in medium								
	Control	0.5	0.7	1.0	1.3	1.5	1.75	2.0	
Spores	++++	++	+++	++++	++++	+++	+++	++	
Vegetative cultures	++++	+++	+++	++++	++++	++++	+++	+++	

Test material	Per cent dextrose in medium								
	2.25	2.5	2.75	3.0	3.5	4.0	4.5	5.0	7.5	10.0
Spores	+	+	+	+	G	G	G	G	G	G
Vegetative cultures	++	++	++	++	+	+	±	—	—	—

[a] The following symbols are used:
+ Slight growth.
++ Fair growth.
+++ Good growth.
++++ Heavy growth.
± Doubtful.
— No evidence of growth.
G Slight germination of spores.

EXPERIMENTAL PROCEDURE, USING PLATE CULTURES

The egg-yolk suspension agar is not entirely satisfactory for counting colonies in plate cultures, since the egg yolk gives the medium a cloudy, semiopaque appearance. However, by using the supernatant fluid from the egg-yolk suspension or a somewhat smaller amount of the suspension for each tube of yeast extract agar (10 to 15 drops), a fairly satisfactory plate culture is obtained if the proper amount of inoculum is used. The following procedure is used: To a series of melted tubes of yeast-extract agar containing varying amounts of dextrose as described above (Table II) the egg-yolk suspension is added and the desired inoculation of the tube made while the medium is still liquid. The tubes are agitated to mix the contents thoroughly and then poured into sterile Petri dishes.

These on cooling are inverted and incubated for 48 hours at 37° C., after which counts are made. If the plates are flooded with a dilute solution of fuchsin or eosin before counting, the colonies are more easily differentiated for counting in the semiopaque medium. Vegetative cultures only were used for plating. A suspension of one loopful of culture in 3 cc. of sterile broth is made and one loopful of that is used to inoculate each plate. Dilution in sterile water was also tried, using 1 cc. of the dilution for each plate, but without success, since there seems to be a minimum amount of initial inoculum required, below which it is difficult to obtain growth.

TABLE II.—*Average number of colonies per 4-mm. loopful of vegetative culture suspension in broth on plates of varying sugar concentration*

Per cent dextrose	Average number of colonies	Per cent dextrose	Average number of colonies
Control (o)	1,500	2.0	
0.5	1,590	2.5	150
1.0	1,560	3.0	0
1.5	914	3.5	0

OBSERVATIONS

SPORE GERMINATION AND GROWTH IN RELATION TO SUGAR CONCENTRATION

At different times during the investigation seven different series of culture tubes were made, using as material for inoculation either scales or, in most cases, ropy remains heavily laden with spores of *Bacillus larvae*, but no vegetative rods. This material was taken from six different samples of diseased brood from different localities. From these series of cultures, varying in sugar concentration from 0.5 per cent to 10 per cent dextrose, it was found that active growth occurs up to and including 2.5 per cent dextrose, although some growth occurs occasionally up to 3 per cent (Table I). The exact limits varied slightly with different strains as well as with variation in the amount of inoculum. Even up to 10 per cent dextrose concentration, a varying small number of spores germinate, as is demonstrated by stained smears, but they give no further evidence of vegetative growth upon the culture medium.

GROWTH FROM ACTIVE VEGETATIVE CULTURES

In a similar manner five different series of tubes with varying sugar concentrations were made, using 24-hour cultures of three different characteristic vegetative cultures of *Bacillus larvae*, previously isolated and accustomed to growth on artificial culture media for different lengths of time. Good growth occurs on the average up to 2.5 per cent to 3 per cent dextrose concentration, with evidence of varying slight growth up to 4 per cent and in one case up to 4.5 per cent (Table I). In the latter case much of the variation is due to variation in the amount of initial inoculum. If a heavy inoculation is made on the surface of the agar tubes, the upper sugar concentration limits for inhibition of growth are increased, although in these cases the growth was meager at best. Stained smears, however, made after a few days, from the higher sugar concentrations particularly, soon showed the peculiar disintegration of the rods noted by White (*55*) as taking place in old cultures and where spore formation is inhibited, such as in the presence of sugar. This, according to observations of Sturges and Rettger (*44*) on other organisms, suggests that this disintegration of the rods is the result of autolysis.

QUANTITATIVE GROWTH IN PLATE CULTURES

Great difficulty is found in obtaining satisfactory plate cultures. Only two series of plate cultures were obtained which could be counted successfully. The average number of colonies showed a definite decrease with increased sugar concentration, with no growth at 3 per cent or higher. (Table II, fig. 10). As stated above, the plate method, with the small amount of initial inoculum necessary for accurate counts, is not a satisfactory method for obtaining growth of *Bacillus larvae* under these conditions, although the method may be used for obtaining pure cultures.

From these observations (Table I) it is, therefore, safe to conclude that a concentration of reducing sugar of approximately 3 to 4 per cent or more inhibits the growth of *Bacillus larvae*, although slight germination of spores may take place at higher sugar concentrations.

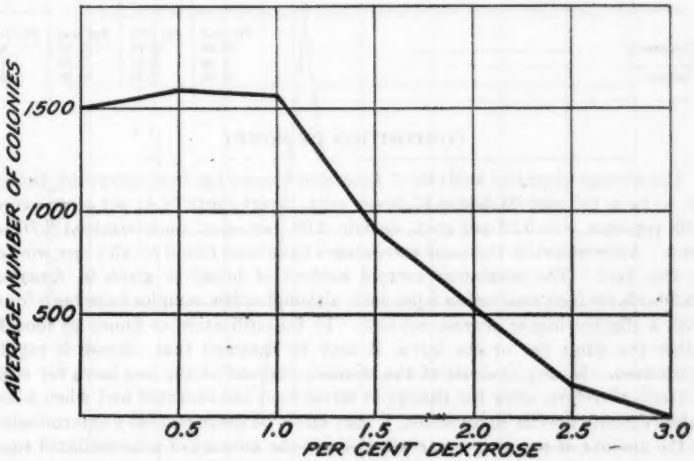

FIG. 10.—Average number of colonies per 4-mm. loopful of vegetative culture suspension with varying sugar concentration (Table II)

QUANTITATIVE DETERMINATION OF UNASSIMILATED SUGAR IN THE LARVAL INTESTINE AT VARIOUS AGE PERIODS

In the preliminary experiments it was shown that unassimilated sugar is present in the intestinal contents of the actively feeding larva, which apparently is assimilated completely by the time the prepupa has extended in the cell and has become quiescent. Since it is demonstrated that a direct relation exists between the growth of *Bacillus larvae* in suitable culture media and its reducing sugar concentration, it is now necessary to determine quantitatively the amount of unassimilated sugar in the intestine of the feeding larva and in the intestine of the prepupa, in order to determine whether reducing sugar concentration has any bearing on the time of attack by American foulbrood.

COMPOSITION OF BROOD FOOD

The older bee larvæ (*40*) receive a food consisting of a mixture of honey or nectar and pollen, rich in sugar, chiefly reducing sugar. This sugar constitutes about 45 per cent of the dried substance, or over 13 per cent of the fresh substance. The food of younger larvæ contains only about 5 per cent of sugar in the fresh

material (Table III, fig. 11). Nelson and Sturtevant (*35*) and Lineburg (*28*) have shown that the change in the composition of this food comes definitely soon after the second day, instead of the fourth day, as stated by Von Planta, after which increasingly large amounts of honey and pollen are fed up until the time of sealing. The larva is fed during this period about as fast as it can ingest the food. From this it is reasonable to suppose that there must be a constant surplus of unassimilated food in the larval intestine until after feeding has ceased.

TABLE III.—*Percentage composition of worker brood food, calculated from Von Planta (40), and on the basis of his assumption of 70 per cent water content*

Substance	Under four days		Over four days	
	Dried substance	Fresh substance	Dried substance	Fresh substance
	Per cent	*Per cent*	*Per cent*	*Per cent*
Nitrogenous	53.38	16.01	27.87	8.36
Fat	8.38	2.51	3.69	1.11
Glucose	18.09	5.43	44.93	13.48

COMPOSITION OF HONEY

The average chemical analysis of American honeys has been shown by Browne (*9*) to be as follows: Moisture 17.59 per cent, invert sugar 74.41 per cent, sucrose 1.98 per cent, ash 0.23 per cent, dextrin 2.09 per cent, undetermined 3.70 per cent. Approximately the same percentages have been found by all other workers in this field. The maximum sucrose content of honey is given in American standards for food analysis as 8 per cent, although a few samples have been found with a slightly higher sucrose content. In the utilization of honey as food by either the adult bee or the larva, it may be assumed that sucrose is rapidly hydrolized. In any analysis of the stomach content of the bee larva for sugar content, therefore, after the change in larval food has occurred and when honey enters directly into its composition, it may safely be assumed that a determination of the amount of reducing sugar will indicate the amount of unassimilated sugar in the intestine, since there will be but a small additional sugar content from sucrose, if any of the latter sugar still remains. In determining the sugar content of the whole larva, as was done in most of the present work, it may be assumed that there is a comparatively small amount of reducing sugar in the blood stream, because of the exceedingly rapid transformation of these sugars into fat and glycogen which are known to occur in the bee larva. It is therefore concluded that the sugar found in the whole larva is virtually that which occurs in the intestine alone, and this greatly simplifies the work of analysis.

COMPOSITION OF THE LARVA AT DIFFERENT AGE PERIODS

The work of Straus (*43*) on the chemical composition of the worker and drone brood during their different developmental stages gives the results of the metabolism of this food, as indicated by the presence of fat and glycogen stored in the so-called fat body of the larva (Table IV, fig. 12). He was unable to demonstrate more than a trace of what he terms reducing substances, except in one case in which only a slight amount was found. He believes that this is because the sugar of the larval food is assimilated so rapidly, as is indicated in the larval composition by the exceedingly rapid increase in the amount of glycogen and fat until after feeding has ceased.

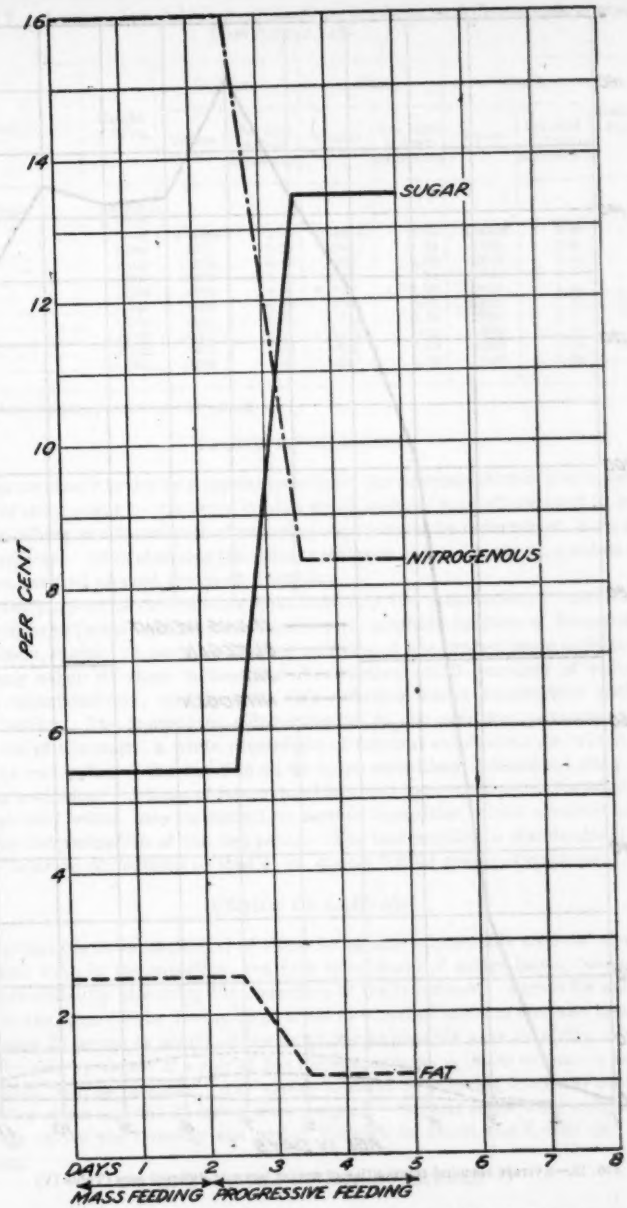

FIG. 11.—Per cent composition of worker brood food (Table III)

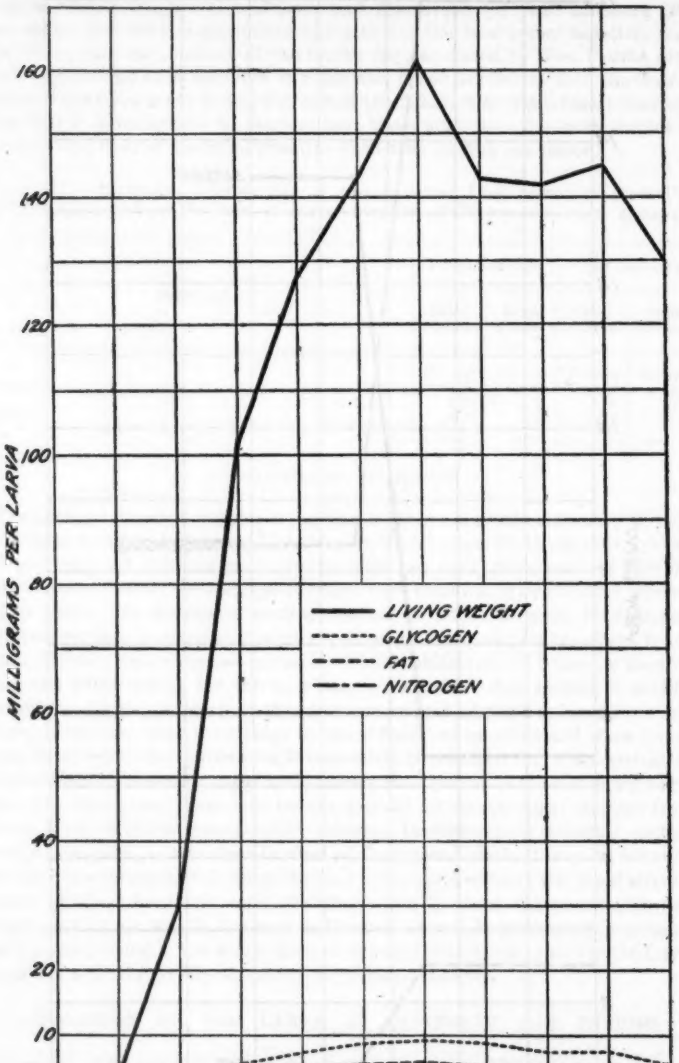

FIG. 12.—Average chemical composition of worker larvæ at different ages (Table IV)

TABLE IV.—*Average chemical composition of worker larvæ at different ages, compiled from Straus (43)*

Age	Weight of larva	Glycogen		Fat		Nitrogen		Reducing sugar
		Grams per larva	Per cent of fresh substance	Grams per larva	Per cent of fresh substance	Grams per larva	Per cent of fresh substance	
Days	*Grams*							
1	0.00030							
2	.00340	0.00008	2.50	0.00004	1.53	0.00009	2.86	
3	.03000	.0012	2.75	.00005	1.64	.0005	2.04	
4	.10010	.0055	5.58	.0031	3.60	.0016	1.44	
5	.12775	.0072	5.57	.0047	3.64	.0016	1.47	0
6	.14290	.0088	6.95	a.0057	a3.98	.0019	1.45	
7	.16140	.0092	6.43	.0060	3.71	.0018	1.22	Trace.
8	.14300	.0089	6.35	.0051	3.53	.0027	1.51	Trace.
9	.14200	.0075	5.21	.0052	3.66	.0022	1.60	
10	.14500	.0075	5.24	.0049	3.60	.0022	1.58	0.0002
11	.13000	.0056	4.21	.0047	3.26	.0023	1.68	

a Calculated by interpolation and averaging.

CHOICE OF REAGENT

It was necessary to devise a special technic for the determination of the unassimilated reducing sugar in the larva by the application of procedures used in other analyses where small amounts of reducing sugars must be determined, such as in urine analysis. After studying the various methods of sugar analysis, a volumetric titration method seemed the most promising.

For the purpose of determining quantitatively the unassimilated sugar in the bee larva at different ages, the modified copper sulphate solution of Benedict (*5*) was chosen, mainly because, as in urine analysis, it has proved more satisfactory than any other titration method for determining small amounts of reducing sugars quantitatively, and because this solution keeps indefinitely without deteriorating. The potassium sulphocyanate in the solution produces, upon reduction of the sugar, a white precipitate of cuprous sulphocyanate, which permits the end point of the reaction to be more accurately determined than with Fehling's solution. A trace of ferrocyanid is added to prevent precipitation of red cuprous oxid which may be caused by certain impurities, which would interfere with the determination of the end point. The test solution is standardized to a known solution of dextrose so that 5 cc. equals 0.0102 grams of dextrose.

CHOICE OF LARVAE

Since there is little likelihood of there being any appreciable amount of sugar elsewhere than in the intestine, analyses were made of entire larvæ, because of the great difficulty attending the dissection of the intestines. Larvæ for analysis were chosen from combs having large areas of brood of uniform size and age. In most cases 25 larvae as nearly of the same size as possible were carefully removed from the cells by means of a pair of fine forceps, care being taken to remove as little uningested food as possible. Any visible amount of adhering food was removed with filter paper and the 25 larvæ were weighed. Several series were weighed for each age above the two-day age period through to about the fourth day after capping.

DETERMINATION OF AGE OF LARVAE

When choosing larvæ for the analysis, the approximate age was determined by comparison with drawings to scale by Nelson and Sturtevant (*35*) of larvæ of known age at various age periods, 24 hours apart. Nelson and Sturtevant, as

well as Straus (Table IV), also give weights for larvæ of known age, but in order to eliminate the danger of variations due to the effect of different seasonal and environmental conditions, the average age of the larvæ analyzed from various groups of 25 was determined by comparison with a series of weighings of larvæ of known age that were made during this same period (*35*). The various series of weights, with the corresponding determinations of reducing sugar, were arranged in age groups, 24 hours apart, as shown in Table V. In some cases, such as the small two-day larvæ, or the quiescent prepupæ, where the amount of unassimilated sugar is small, 50 larvæ were taken for analysis, but usually 25 proved satisfactory.

PREPARATION OF MATERIAL FOR ANALYSIS

Several difficulties were encountered in the preparation of material for sugar determination. At first, attempts to extract the sugar were made by macerating the larvæ with distilled water and filtering through filter paper. This produced a cloudy opalescent liquid, indicating the presence of colloidal material, and this solution did not give the characteristic reaction with the Benedict reagent. Various clarification methods were tried. Precipitation with both neutral and basic lead acetate (*10, p. 276*) solutions proved unsatisfactory, something still remaining to interfere with the reaction. Mercuric nitrate solution, which is sometimes used to clarify liquids of animal origin such as blood, urine, and milk, was tried (*10, p. 447*). This method occasionally gave good results, mainly with the younger larvæ, but often with older larvæ and prepupæ the colloidlike material still remained in the filtrate, interfering with the reaction. Furthermore, because of the numerous filtrations necessary to remove successive precipitates, it was feared that more or less sugar is lost by adsorption to those precipitates, even with careful washing. An attempt was made to clarify by filtration with suction through a celloidin membrane, and this gave a clear solution which reacted well with the test solution, but the method required too great time. The method finally adopted was by extraction with 50 per cent alcohol, similar to the method used in the extraction of sugars from grains and similar products (*11*). This method proved successful, since the alcohol causes precipitation of all solid matter, giving a clear filtrate which reacted properly with the Benedict's reagent. Since glycogen in water solution is colloidal in nature, and thereby difficult to remove by filtration from such a solution, it is doubtless the glycogen present in the larva which prevented clarification and interfered with the reaction. It is possible for this reason that Straus (*43*) failed to demonstrate reducing sugars. To determine this point, a small amount of glycogen was added to a known solution of dextrose and tested with the copper sulphate solution, and the known reducing sugars could not now be demonstrated quantitatively. Since glycogen is insoluble in alcohol (*10, p. 443*) the 50 per cent alcohol precipitates the glycogen and thereby removes materials interfering with the reaction in the filtrate. Even though there may still be a small loss of reducing sugar by adsorption or by some other means, the results obtained are of value for purposes of comparison. If any reducing sugar is lost by the method adopted, the amount is exceedingly small and may therefore be disregarded, since repeated washings failed to demonstrate its presence.

TECHNIC ADOPTED

After weighing, the larvæ are removed to a small porcelain mortar and macerated in 30 cc. of 50 per cent alcohol. This material is then washed carefully into a small flask and allowed to stand from two to three hours before filtering. The precipitate is washed with 50 per cent alcohol. The filtrate is then made up to 50 cc. with distilled water, and run into a burette. Five cc. of the stand-

ardized Benedict's solution are placed in a white porcelain casserole and diluted with an equal amount of distilled water. To this are added about 5 grams of anhydrous sodium carbonate and a small amount of ground pumice. This solution is brought to a boil and the larval extract is run in slowly, drop by drop at the end, until the blue color disappears and a white precipitate forms. From the number of cc. of larval extract used, the milligrams of sugar per larva and the per cent of sugar per larva are calculated (Table V).

TABLE V.—*Unassimilated sugar in intestinal content of larvæ at different ages*

Larvæ of known age, Sturtevant (35)			Larvæ analyzed for presence of unassimilated sugar (weights in grams)										
Age in days	Average weight	Limits by weight for age groups	Date	Weight of sample	Number of larvæ	Average weight of 1 larva	Extract used[a]	Equivalent number of larva	$CuSO_4$ solution	Equivalent dextrose	Dextrose per larva	Sugar per larva
2	*Gram* 0.004745	*Gram* Up to 0.014685.	*1922* 7-18	*Gram* 0.6233	50	*Gram* 0.01247	*Cc.* 50	50	*Cc.* 5	*Gram* No reaction.	*Gram* 0	*P. ct.* 0
3	.024626	0.014685 to 0.059308.	7-25	.4967	25	.01987	50	25	5	No reaction.	0	0
			7-25	1.1072	25	[b].04429	44	22	5	0.01020	0.000463	1.13
			8-2	[b]1.0979	25	[b].04392	23.5	23.5	5	.01020	.000434	.98
			5-9	[c]2.2906	50	.04581	90	45	10	.02040	.000453	.94
			8-11	1.1706	25	.04682	42	21	5	.01030	.000490	1.04
			7-27	1.4009	25	.05604	20.25	10.125	5	.01020	.001007	1.79
	Average					.043222					.000475	.98
4	0.003990	0.059308 to 0.120369.	8-2	1.6749	25	.06700	21	10.5	5	.01020	.00097	1.44
			8-11	[d]1.6817	25	.06727	11	9.16	5	.01030	.00112	1.66
			8-11	1.9372	25	.07749	12.75	6.375	5	.01030	.00161	2.07
			8-11	1.9916	25	.07966	18.1	9.05	5	.01030	.00113	1.41
			7-31	2.3044	25	[b].09218	6.8	3.4	5	.01020	.00300	3.25
			8-2	2.3566	25	[c].09426	8.6	4.3	5	.01020	.00237	2.51
			8-30	2.3733	25	.09493	6.58	3.29	5	.01030	.00313	3.29
			7-25	2.4901	25	.09960	5.8	2.9	5	.01020	.00351	3.52
			8-2	2.6748	25	.10699	8.0	4.0	5	.01020	.00255	2.37
			8-17	2.6843	25	.10737	4.75	2.38	5	.01030	.00431	4.01
			8-18	2.7781	25	.11112	4.35	2.175	5	.01030	.00473	4.25
			8-2	2.7919	25	.11168	7.0	3.5	5	.01020	.00291	2.61
			8-18	2.8205	25	.11282	4.35	2.175	5	.01030	.00473	4.19
			6-1	2.8332	25	.11333	12.7	6.35	8.9	.018156	.00286	2.52
			8-11	2.8972	25	.11589	6.8	3.4	5	.01030	.00303	2.61
			8-4	2.9274	25	.11710	7.1	3.55	5	.01020	.00287	2.45
			8-2	2.9505	25	.11802	7.1	3.55	5	.01020	.00287	2.43
			8-31	2.9749	25	.11900	5.2	2.6	5	.01030	.00396	3.32
			8-2	2.9908	25	.11963	6.0	3.0	5	.01020	.00340	2.84
			8-17	3.0038	25	.12015	4.5	2.25	5	.01030	.00457	3.80
			8-4	3.0105	25	.12042	6.5	3.25	5	.01020	.00314	2.61
	Average					.10314					.00290	2.82
5	0.146748	0.120369 to 0.150676.	6-1	[c]3.0961	25	.12384	27.0	6.75	10	.02040	.00317	2.57
			8-18	3.1148	25	.12459	4.2	2.1	5	.01030	.00490	3.93
			8-18	3.1953	25	.12781	4.2	2.1	5	.01030	.00490	3.83
			8-11	3.2141	25	.12856	6.4	3.2	5	.01030	.00322	2.51
			7-26	3.3153	25	.13261	5.6	2.8	5	.01020	.00364	2.75
			7-31	[e]3.3278	25	.13311	5.0	2.5	5	.01020	.00408	3.06
			8-11	3.3479	25	.13392	6.5	3.25	5	.01030	.00317	2.36
			8-10	3.3602	25	.13441	6.15	3.075	5	.01030	.00334	2.45
			7-31	3.3689	25	.13476	4.1	2.05	5	.01020	.00497	3.69
			8-11	3.3706	25	.13482	6.1	3.05	5	.01030	.00337	2.49
			7-27	3.3721	25	.13488	4.5	2.25	5	.01020	.00453	3.35
			7-25	3.4029	25	.13612	3.4	1.7	5	.01020	.00600	4.41
			7-25	3.4620	25	.13848	5.25	2.625	5	.01020	.00388	2.80
			8-17	3.4644	25	.13858	3.5	1.75	5	.01030	.00588	4.23
			8-31	3.4775	25	.13910	4.6	2.3	5	.01030	.00448	3.22
			7-25	[f]3.6156	25	.14462	7.92	3.96	6.07	.01238	.00312	2.15
			8-17	3.6394	25	.14558	3.6	1.8	5	.01030	.00572	3.92
			8-17	[f]3.6971	25	.14788	4.2	2.1	5	.01030	.00490	3.31
			8-18	[f]3.7164	25	.14866	5.2	2.6	5	.01030	.00396	2.66
	Average					.13591					.00428	3.14

[a] Unless otherwise stated, total cc. of extract equals 50.
[b] Total extract, 25 cc. only.
[c] Total extract, 100 cc.
[d] Total extract, 30 cc. only.
[e] Just sealed, early.
[f] Just sealed, still coiled.

TABLE V.—*Unassimilated sugar in intestinal content of larvæ at different ages*—Continued

Larvæ of knownage, Sturtevant (55)			Larvæ analyzed for presence of unassimilated sugar (weights in grams)									
Age in days	Average weight	Limits by weight for age groups	Date	Weight of sample	Number of larvæ	Average weight of 1 larva	Extract used	Equivalent number of larva	$CuSO_4$ solution	Equivalent dextrose	Dextrose per larva	Sugar per larva
	Gram	*Gram*	*1922*	*Grams*		*Gram*	*cc.*		*cc.*	*Gram*	*Gram*	*P.ct.*
6	0.155005	0.150876 to maximum and down to 0.148326.	7–18	[c] 3.8012	25	0.15205	18.05	4.51	9.6	0.19584	0.00434	2.85
			8–31	3.8038	25	.15215	5.4	2.7	5	.01030	.00381	2.50
			8–11	3.8925	25	.15570	4.35	2.175	5	.01030	.00473	3.03
			8–31	[a] 3.9783	25	.15913	6.0	3.0	5	.01030	.00343	2.15
			8–31	4.1249	25	.16500	7.0	3.5	5	.01030	.00294	1.78
			7–27	3.7706	25	.15062	6.65	3.33	5	.01020	.00306	2.03
	Average					.15581					.00372	2.39
7	0.141648	0.148326 to 0.139406.	8–18	3.6980	25	.14792	7.3	3.65	5	.01030	.00282	1.91
			8–10	3.5600	25	.14280	8.9	4.45	5	.01030	.00231	1.56
			8–10	[b] 3.5835	25	.14334	11.5	5.75	5	.01030	.00179	1.25
			8–4	3.5572	25	.14229	7.4	3.7	5	.01030	.00275	1.93
			8–18	[d] 3.4871	25	.13948	45.0	22.5	5	.01030	.00050	.36
	Average					.14397					.00203	1.40
8	0.137165	0.139406 to 0.135158.	7–26	3.4358	25	.13743	50.0	25	5	No reaction.	0	0
			7–18	[f] 3.4453	25	.13781	50.0	25	5	No reaction.	0	0
	Average					.13762					0	0
9	0.133152		8–4	[h] 3.3232	25	.13293	50.0	25	5	No reaction.	0	0

[c] Total extract, 100 cc.
[a] All sealed, coiled or with backs out. Feeding ended and spinning of cocoons started.
[b] Cocoon partially spun, still some color in the intestine.
[d] Cocoon not quite finished, still moving somewhat, no color in intestine.
[f] Quiescent prepupæ, intestines colorless, empty, histolysis started.
[h] First indication of change in external form.

OBSERVATIONS

Over 60 samples of 25 larvæ each of various ages, containing over 1,600 individual larvæ, were analyzed for the presence of reducing sugars. The largest number of analyses were made on larvæ from 3½ to 5½ days of age during the active honey and pollen feeding period. At least five analyses were made of each of the other age periods which might show the presence of sugar. To obtain averages with a small probable error, the analyses are grouped by age periods of 24 hours each, as described earlier (Table V, fig. 13). All larvæ in the two-day group, as well as one sample of larvæ nearly as heavy as the three-day average larva, showed no reducing sugar. Larvæ in the three-day group, averaging 0.043222 gm. in weight, gave 0.000475 gm. of reducing sugar per larva, or 0.98 per cent concentration. Larvæ in the four-day group, averaging 0.10314 gm. in weight, gave 0.00299 gm. of reducing sugar per larva, or 2.82 per cent concentration. Larvæ in the five-day group, comprising those just prior to sealing, with a few just sealed, averaging 0.13591 gm. in weight, gave 0.00428 gm. of reducing sugar per larva, or 3.14 per cent concentration. In the five-day group there were two samples which gave a concentration of over 4 per cent, the maximum being 4.41 per cent. The six-day group, comprised entirely of larvæ that had been sealed, had finished feeding and had started spinning, averaging 0.15581 gm. in weight, gave 0.00372 gm. of reducing sugar per larva, or 2.39 per cent concentration. This group contains larvæ of maximum size (fig. 14). From

this point on the gross weight decreases as preparation for metamorphosis begins. The seven-day group, comprising larvæ which are still moving about in spinning, and most of which show only a slight remaining color in the intestines, indicating

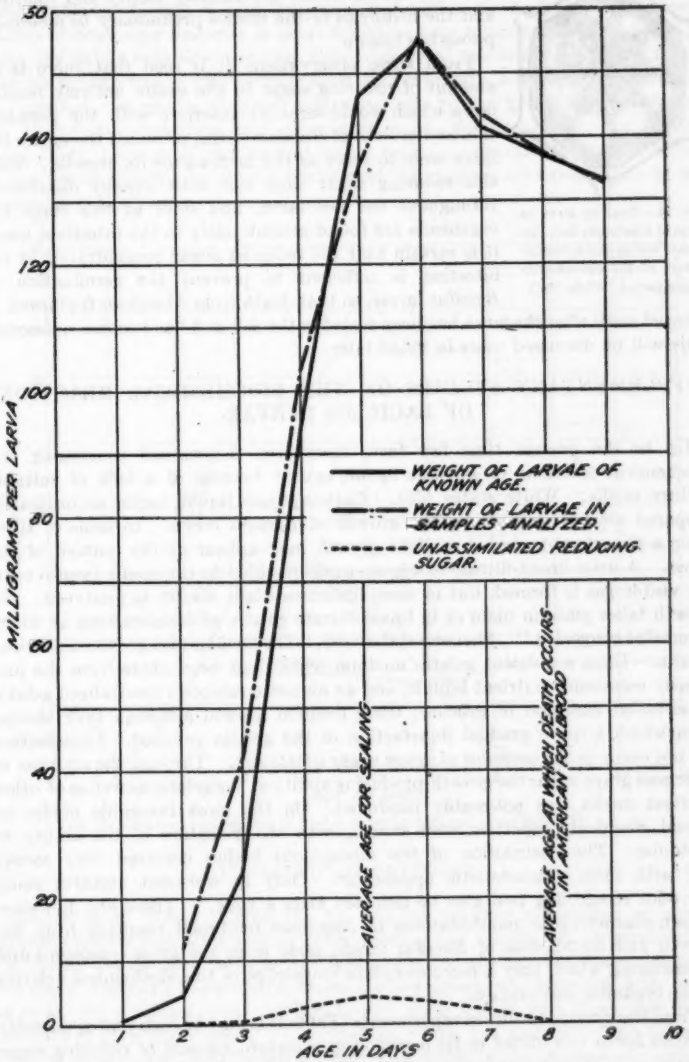

FIG. 13.—Unassimilated sugar in larvæ at different ages (Table V)

that the connection between ventriculus and end gut is made, averaging 0.14397 gm. in weight, gave 0.90203 gm. of reducing sugar per larva, or 1.40 per cent concentration. One sample in this group gave as low as 0.36 per cent. Larvæ

of the eight-day group, averaging 0.13762 gm. in weight, showed a total absence of reducing sugar. These larvæ represent the two-day quiescent prepupal stage (fig. 3 and 4). They have stretched out motionless in the cell, the intestines are entirely empty and colorless, and the histolysis of the tissues preliminary to metamorphosis has begun.

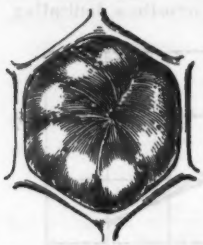

FIG. 14.—Healthy larva at age of maximum size, just after sealing and before the start of the cocoon-spinning period (White (56))

From these observations it is seen that there is an amount of reducing sugar in the entire actively feeding larva which would seriously interfere with the germination and growth of *Bacillus larvae*, provided the entire bee larva were to serve as the medium for its growth. Since this reducing sugar does not exist equally distributed throughout the bee larva, and since at this stage the organisms are found almost solely in the intestinal tract, it is certain that the reducing sugar concentration of the intestine is sufficient to prevent the germination of *Bacillus larvae*, so that death from American foulbrood is delayed until after the larva has been sealed in the cell and has become quiescent. This will be discussed more in detail later.

SUPPLEMENTARY STUDIES ON THE BIOCHEMICAL REACTIONS OF BACILLUS LARVAE

Up to the present time few facts have been determined concerning the biochemical reactions of *Bacillus larvae*, mainly because of a lack of suitable culture media. White states (*55*), "Carbohydrate liquid media as ordinarily prepared are not suitable for the growth of *Bacillus larvae*. In some of these after a considerable period a slight growth may appear at the bottom of the tubes. A little brood-filtrate or egg-suspension added to the media improves it. No visible gas is formed, but in some instances slight acidity is produced. No growth takes place in plain or in brood-filtrate gelatin at temperatures at which it remains congealed." Maassen states (*28*), "The bacillus also grows on nutrient gelatin. Upon a nutrient gelatin medium which had been made from the previously mentioned nutrient liquids, and an almost completely neutralized gelatin (a so-called emulsion of gelatin), there resulted growth although very slowly, from which a quite gradual liquefaction of the gelatin resulted. Liquefaction did not occur in the presence of grape sugar (dextrose). Through the addition of 1 per cent grape sugar the growth-producing ability of the gelatin as well as of other nutrient media was noticeably improved. On the most favorable media no special chemical properties were shown, with the exception of the ability to peptonize. The destruction of the albuminous bodies occurred very slowly and with little characteristic appearance. Only in worn-out cultures could any odor resembling foul glue be detected after a time." There are, however, certain characteristic manifestations in American foulbrood resulting from the growth and metabolism of *Bacillus larvae*, aside from the gross symptoms and appearances, which only a more complete knowledge of the biochemical activity of the organism can explain.

From the previous cultural experiments (Table I) it may be seen that apparently *Bacillus larvae* can utilize in its metabolism a certain amount of reducing sugar (dextrose), although this sugar is not necessary to the development of the organism. In the larva which is attacked by American foulbrood there may be two sources of sugar, that present unassimilated in the intestine and that hydrolyzed from the stored glycogen. Hydrolysis of glycogen may occur in connection with histolysis of the tissues preparatory to metamorphosis through enzym

action, or *Bacillus larvae* itself may have the ability to produce enzymes which hydrolyze the glycogen, or it may be a combination of both. Through the utilization of this reducing sugar one would expect that there at least would be a considerable production of acid, but, as stated earlier, the hydrogen-ion concentration of dead ropy material is never found to vary much from P_H=6.6 to 6.8. Since the data available concerning the biochemical reactions of *Bacillus larvae* offer no explanation of this hydrogen-ion concentration, a series of experiments was devised, the results of which add materially to the knowledge concerning the biochemical reactions and relationships of *Bacillus larvae*. In certain cases where, because of the limitations on growth, cultural growth has failed, it was found possible to obtain the desired information by examination of the diseased larval remains.

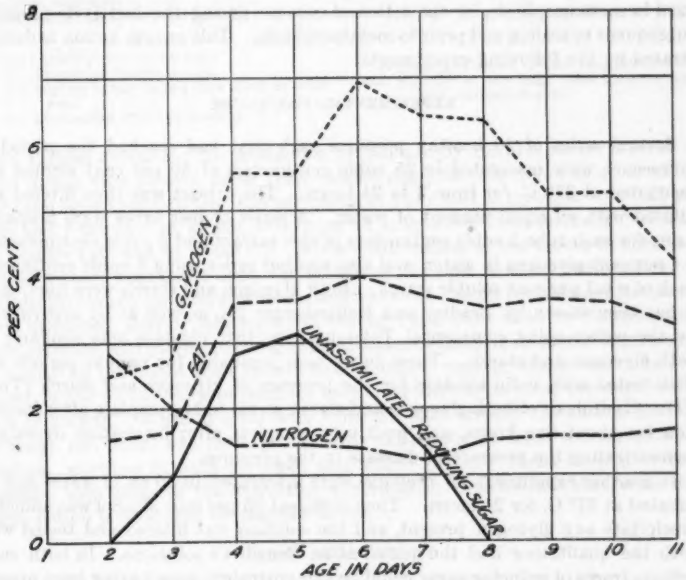

FIG. 15.—Per cent composition of worker larvæ at different ages (Tables IV and V)

UTILIZATION OF GLYCOGEN

According to Straus (Table IV, fig. 15) the greatest percentage of stored glycogen occurs just after sealing, when feeding has ceased. If an emulsion of the tissues of a larva of this age, or slightly older, at the age when prepupæ usually die of American foulbrood, is tested for the presence of glycogen with iodin solution,[4] the resulting deep reddish brown color shows that there are large amounts of glycogen present. If a prepupa which has just died from disease, slimy in consistency, light brown in color, and which in the microscopic picture still shows the presence of vegetative rods, is tested with iodin solution, it will

[4] Glycogen treated with iodin solution gives a color varying from brown to wine red, which disappears upon heating to 60° C. but returns again upon cooling. Soluble plant starch with iodin solution gives the following reactions: Amylodextrin, first dextrin of conversion, dark blue; erythrodextrin, second dextrin of conversion, red; intermediate steps give various shades of purple or lavender.

be found that most of the glycogen has disappeared, although the iodin solution gives a light yellowish brown color. The presence of a trace of reducing sugar also occasionally can be demonstrated with Benedict's solution in diseased material of this type where vegetative organisms are still actively present. In material which has decomposed completely, has reached the dark brown ropy stage (fig. 9), and contains only spores of *Bacillus larvae*, glycogen is found to be completely absent, nor can any reducing sugar be demonstrated, the sugars having been completely destroyed.

This type of material stained with Sudan III or osmic acid (*32, p. 78*) shows fat globules in practically the same condition and amount as in healthy larvæ, so that fat is apparently not acted upon by *Bacillus larvae* even after drying down to the scale stage.

Glycogen of the fat body of the healthy larva is hydrolyzed to dextrose to be used in metamorphosis, by the action of enzyms during the histolytic processes subsequent to sealing and prior to metamorphosis. This enzym action is demonstrated by the following experiments:

EXPERIMENTAL PROCEDURE

Several series of 50 healthy prepupæ each that had reached the period of quiescence were macerated in 25 cubic centimeters of 50 per cent alcohol and incubated at 37° C. for from 3 to 24 hours. The extract was then filtered and diluted with an equal amount of water. A series of test tubes were prepared, using for each tube 5 cubic centimeters of this extract and 5 cubic centimeters of 0.4 per cent glycogen in water, and also another series using 5 cubic centimeters each of a 0.1 per cent soluble starch. Both glycogen and starch were used, since it has been shown by Bradley and Kellersberger (*3*), as well as by experiments by the writer using commercial Taka-diastase, that diastase acts similarly on both glycogen and starch. These tubes were incubated for various periods and then tested with iodin solution for the presence of glycogen and starch (Table VI). Hydrolysis of both glycogen and starch seems to be complete after incubation for about five hours, and positively complete after incubation overnight, demonstrating the presence of diastase in the prepupæ.

In another experiment 50 prepupæ were macerated in 50 cc. of water and incubated at 37° C. for 24 hours. Then sufficient 95 per cent alcohol was added to precipitate any glycogen present, and the solution was filtered and tested with both the qualitative and the quantitative Benedict's solutions. In both cases definite traces of reducing sugar could be demonstrated, none having been present in the original solution before incubation, again demonstrating enzym activity of the larval tissues. This may have been due to action by bacterial contamination, but if such had been the case the sugar would probably have been fermented and could not have been demonstrated.

In a similar manner extracts with 50 per cent alcohol were made of ropy diseased material, enzym activity being demonstrated in the same manner as above. This, however, does not indicate whether the organism causing the disease has any diastatic power or whether the reaction was due to enzyms remaining in the decomposed tissues. Further extracts were made with 25 per cent and 50 per cent alcohol of several 48-hour vegetative cultures of *Bacillus larvae* grown on egg-yolk suspension medium. These extracts showed definite enzym activity with glycogen after a few hours' incubation, and more positive activity after incubation overnight (Table VI), while with starch marked hydrolysis was shown after only a few hours' incubation.

TABLE VI.—*Test for diastatic action with alcoholic extract*[a]

Test material	Control tubes		Color with iodin after incubation of—									
			0 hour		½ hour		2½ hours		5½ hours		18 hours	
	Glycogen	Starch	Glycogen	Starch	Glycogen	Starch	Glycogen	Starch	Glycogen	Starch	Glycogen	Starch
Extract of healthy prepupæ	++++ (brown)	++++ (blue)	+++	++	++	+	+	±	——	——	——	——
Extract of decomposed ropy remains	++++	++++	——	——	++	+	+	±	——	——	——	——
Extract of vegetative cultures	++++	++++	——	——	+++	++	++	+	——	——	——	——

[a] The following symbols are used:
++++ Deep color, brown or blue.
+++ Slightly lighter brown than check or wine color.
++ Light coffee brown or lavender.
\+ Trace faint brown or trace faint lavender.
—— No color or only iodin color, showing complete diastatic action.

To further determine the production of diastase by *Bacillus larvae*, a series of Petri dishes were poured, using yeast-extract egg-yolk suspension agar, to which had been added respectively 0.25 per cent and 1 per cent of glycogen and 0.25 per cent and 1 per cent of starch, this being an adaptation from methods described by Vedder (*48*) and by Allen (*1*). After solidification of the media in the Petri dishes, smears were made upon the surface of the agar from 48-hour cultures of various previously isolated strains of *Bacillus larvae*. After several days the plates were examined, first by holding up to the light and then later by flooding with iodin solution, and comparing with control plates containing no starch or glycogen. In nearly all the plates good growth had occurred, causing clear areas to be produced in the cloudy culture medium extending slightly beyond the edge of the area of growth. When flooded with iodin the halo around the culture growth, although not wide, was more prominently differentiated from the surrounding medium, showing in both glycogen and starch plates. These results, in conjunction with those of the extraction experiments, demonstrate that weak diastatic action is produced by *Bacillus larvae*.

ACID PRODUCTION

It has been shown that there is still an appreciable amount of sugar (reducing sugars in the food remaining in the intestines and dextrose available from glycogen) present in the larva after sealing and in the prepupa at the age when American foulbrood attacks, available for fermentation (Tables IV and V). In the various cultural investigations both by others and by the present writer, there is no evidence of carbon dioxid production. It would be expected, however, that at least some acid would be produced from the bacterial fermentation of these sugars, which is known to be present. To determine this more definitely than heretofore, a culture medium was devised for the qualitative determination of acid production, which gave good vigorous growth of *Bacillus larvae*.

The method used is an adaptation of the method of using agar slants for detecting acid formation, instead of liquid medium, described by Conn and Hucker (*18*), in which the change in reaction can readily be seen. The regulation yeast-extract egg-yolk suspension agar was prepared for this purpose by adding to the yeast extract base before sterilization an indicator in the proper amount both to the plain medium and also to a portion to which was added 1 per cent of dextrose.

Brom thymol blue was first used, as it covers the range of the supposed optimum reaction for *Bacillus larvæ* as described earlier. Baker (*4*) also has shown that brom thymol blue, used in about a 0.0024 per cent concentration in culture media, gives the most desirable color for comparison, without inhibiting acid fermentation. This concentration was obtained by using 12 cc. of a 0.2 per cent alcoholic solution of the indicator per liter. After marked acid production in the dextrose tubes was demonstrated with brom thymol blue, brom cresol purple was used as suggested by Conn and Hucker (*18*) in a 0.001 per cent concentration as a check on the end point. This concentration was obtained by using 8 cc. of a 0.2 per cent alcoholic solution of the indicator per liter. The yeast-extract base, both with and without dextrose, was adjusted so that after the addition of the egg-yolk suspension the final medium would have a primary reaction of approximately P_H=7.2, a definite blue grass green in the case of brom thymol blue and a marked purplish tinge with brom cresol purple, except in one series, where the primary reaction of the plain medium was P_H=7.6. These tubes after being slanted were inoculated as usual, both with vegetative cultures and with diseased material containing spores. The change in reaction was noted after different lengths of incubation, and the final reaction was determined by comparison with standard buffer tubes used in combination with tubes of plain egg-yolk suspension media slanted in the same manner. The approximate increase in hydrogen-ion concentration was determined by this comparison (Table VII).

TABLE VII.—*Acid production by Bacillus larvae*

Culture No.	Brom thymol blue indicator				Brom cresol purple indicator			
	Plain medium		1 per cent dextrose		Plain medium		1 per cent dextrose	
	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated
	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H
9693-1	7.6	6.8	7.2	6.0	[b]	[c]	[d]	5.8
9834-1	7.2	6.6	7.2	6.0				
9834-2	7.6	[a] 7.4	7.2	6.2	[b]	[c]	[d]	6.0
9853	6.8–7.0	±6.6	6.6	6.0				
9857	7.2	6.6–6.8	7.2	6.0–6.2				
9867	7.6	[a] 7.4	7.2	6.4	[b]	[c]	[d]	6.2
9869	7.6	[a] 7.4	7.2	6.0	[b]	[c]	[d]	5.8
9874	7.6	7.0–7.2	7.2	6.0	[b]	[c]	[d]	5.8

[a] Doubtful growth.
[b] Beyond end point, no growth.
[c] No change in color, good growth.
[d] Beyond end point, good growth.
[e] No change in color, no growth.

OBSERVATIONS

Several interesting facts were observed from these experiments. Addition of buffer salts to the media delayed the approach to the final hydrogen-ion concentration reaction somewhat, but eventually practically the same end point was reached. Also, in one series of media in which the plain medium was adjusted to about P_H=7.6, little if any growth occurred in these tubes except with two strains of *Bacillus larvae*, indicating that the alkaline limit for growth is about at this point. In cases where the initial reaction of the plain medium was P_H=7.2, the final reaction averaged P_H=6.6 to P_H=6.8 (Table VII). In the case of the medium to which 1 per cent dextrose had been added, the final reaction averaged about P_H=6.0 for brom thymol blue and from P_H=5.8 to P_H=6.0 for brom cresol purple (Table VII). While, therefore, only a slight change in reaction occurred in media without sugar, a marked production of

acid was indicated in the tubes to which 1 per cent dextrose had been added. The maximum production of acid, however, required approximately 48 hours or more, the fermentation of the sugar apparently being relatively slow. As has been stated, however, the reaction of diseased material in various stages of decomposition and drying down is never found to reach a hydrogen-ion concentration of more than $P_H = 6.6$, and usually averages $P_H = 6.8$.

PROTEIN DECOMPOSITION

It is known that certain organisms have the ability to break down protein material under proper conditions, with the production of amino acids and alkaline decomposition substances, which latter tend to neutralize any acid produced from fermentation of sugar. If it can be shown that *Bacillus larvae* has this ability, it will explain the fact that the remains of larvæ dead from American foulbrood do not show a greater acid reaction resulting from the fermentation of the sugar of the intestinal contents. A series of experiments was devised to demonstrate whether such is the case with *Bacillus larvae*.

The prepupa at the age attacked by American foulbrood contains nitrogenous substances as shown by the Kjeldahl nitrogen determination equivalent to 1.45 per cent nitrogen (*43*). The source of this nitrogen is mainly albuminous material, one of the constituents of the larval fat body. Its exact composition has not been determined, but without doubt it is complex in nature. There are certain color reaction tests by means of which the constitution of this nitrogenous material may be indicated.

A delicate test for the presence of coagulable protein is that of Heller (*32, p. 1067*). A suspension of healthy prepupæ in water, treated by pouring about 4 cc. of concentrateed nitric acid down the side of the inclined test tube, causes a white ring to form at the junction of the two liquids. Decomposed ropy material tested in this way gives no indication of such a ring, indicating that the complex protein has disappeared.

One of the most characteristic reactions for complex protein is the biuret test (*32, p. 915*). If some healthy prepupæ are suspended in a few cubic centimeters of 10 per cent sodium hydroxid and are treated with a few drops of a 0.5 per cent copper sulphate solution, a distinct pinkish-violet color is formed, again indicating the presence of complex protein material. Decomposed ropy material tested in this way gives no indication of this color, again indicating the complete disappearance of the complex protein.

There is also the xantho-proteic reaction (*32 p. 916*), which is given both by solid and by dissolved protein, and indicates the presence of the amino-acids, tryptophan, tyrosin, or phenylalanin in the protein molecule, or in solution. Tryptophan gives the reaction most intensely. Both healthy prepupæ and ropy material, boiled with concentrated nitric acid, produce a lemon-yellow color which on cooling and neutralizing with sodium hydroxid changes to an orange, denoting a positive reaction.

An even more delicate reaction for protein is that with Millon's solution (*32, p. 916*). A few cubic centimeters of a suspension of healthy prepupæ, treated with a few drops of Millon's reagent and boiled, cause a brick-red precipitate to form, leaving the liquid practically clear. A solution of decomposed ropy material, treated in the same way with Millon's reagent and boiled, causes a somewhat similar reddish precipitate, but the solution is also distinctly colored similarly, indicating that the protein has been changed in some way, part at least being soluble in water. Tyrosin is the only amino acid in protein that gives this reaction.

Since tryptophan is probably one of the principal constituents of the protein molecule in the healthy prepupa as well as in solution in diseased material, certain tests were made to determine its presence, because this amino-acid is easily utilizable by bacteria and gives decomposition products indicating the nature of bacterial action. The following tests are specific tryptophan reactions:

Adamkiewicz reaction (*32, p. 917*).—A suspension of healthy prepupæ or of diseased material in glacial acetic acid, treated by pouring concentrated sulphuric acid down the side of the inclined tube, causes a violet ring to form at the junction of the two liquids, indicating the presence of tryptophan, either as part of the complex molecule or in solution.

Rhodes reaction (*41*).—To a suspension of healthy prepupæ or of diseased material in water, a few drops of a weak solution of dimethyaminobenzaldehyde is mixed and concentrated sulphuric acid poured down the side of the inclined tube. This produces a violet ring at the junction of the two liquids which, if shaken, produces a reddish violet coloration in the mixture.

PROTEIN DECOMPOSITION PRODUCTS

It is therefore evident that the composition of the nitrogenous material in the healthy prepupæ is more or less complex but that certain amino-acids are available for bacterial metabolism, or are produced as a result of bacterial action.

In the decomposition of nitrogenous material, however, certain bacteria have the power of breaking down these amino-acids, such as tryptophan, to more simple compounds, some of them alkaline in nature, and often more or less foul smelling, or even to break them up into ammonia, the final product of nitrogenous decomposition. Indol is one of the products of such action of bacteria on material containing tryptophan. Its determination is largely used in the characterization of various organisms (*36*). Two indol tests were used, Ehrlich's aldehyde test (*19*) and the vanillin test (*19*), using for both suspensions of diseased material as well as cultures. Test of suspensions of diseased material gave positive results for the presence of indol, both with the Ehrlich method and even more definitely with vanillin. For testing in pure culture a broth consisting of 2 per cent peptone, 10 per cent yeast extract, and a few cubic centimeters of egg-yolk suspension was inoculated, incubating at 37° C. for about one week. Growth took place in this broth sufficiently to give a slight positive pink color with the Ehrlich aldehyde test, increasing on standing, and a much more positive result with the vanillin test.

AMMONIA PRODUCTION

Test of a suspension of diseased material as well as some of the above culture broth with Nessler's reagent (*32, p. 1084*) for presence of ammonia gave indications, from the resulting slight production of characteristic yellowish color, that the decomposition had passed even to the ammonia stage. A more delicate qualitative test was devised, using the modification of the microchemical method of Folin and McCallum (*32, p. 1093*) for the determination of urinary ammonia as described by Steel (*42*). To 25 cc. of a suspension of diseased material, or to broth culture similar to the above, 1 gram of sodium hydroxid and 15 grams of sodium chlorid are added and ammonia-free air bubbled through into 20 cc. of an approximately N/20 sulphuric acid, to which 10 drops of the indicator thymol blue are added. This showed the sulphuric acid solution to have a primary hydrogen-ion concentration of about $P_H=2$. After bubbling air through for an hour or more, in the case of the decomposed ropy material, sufficient ammonia had been carried over to neutralize part of the acid and change the hydrogen-ion concentration reaction from $P_H=2$ to $P_H=2.8$ or 3. Also one culture out of

three showed a change from $P_H=2$ to $P_H=2.8$. Therefore apparently *Bacillus larvae* has the ability of producing at least small amounts of ammonia. It seems probable that the rather pungent volatile gluelike odor often associated with American foulbrood receives some of its characteristics from this ammonia as well as from certain of the protein digestion products.

GELATINE LIQUEFACTION

The ability of putrefactive bacteria to liquefy gelatin is difficult to demonstrate with *Bacillus larvae* because of the cultural limitations. Maasen states (*28*) that slow liquefication takes place, while White (*55*) was unable to demonstrate any growth in gelatin. The writer inoculated a number of tubes of plain gelatin with several strains of *Bacillus larvæ*, all of which showed slight growth, and one or two showed a slight softening of the gelatin about the culture growth. Tubes of gelatin to which some egg-yolk suspension was added showed this softening more markedly, but in no case was there sufficient liquefication to enable one to say that it was positive. Decomposed ropy material inoculated into plain gelatin, on the other hand, gives a marked liquefaction in a short time. This, however, probably is due not to enzyms produced by *Bacillus larvae* so much as to enzyms from the body tissues functioning in the histolysis previous to metamorphosis.

This series of experiments, however, demonstrates that sufficient alkaline decomposition products are formed by the action of *Bacillus larvae* in the prepupa to neutralize most of the acid formed by the fermentation of the sugar in the intestinal contents and the dextrose resulting from the hydrolysis of the stored glycogen.

DISCUSSION

PER CENT CONCENTRATION OF SUGAR

In the data presented it may be seen that there is not an exact correlation between the percentage of dextrose which inhibits the germination or prevents the growth of *Bacillus larvae*, and the percentage of unassimilated sugar in the larva as expressed. The reason for this is that the percentage of unassimilated sugar is calculated in relation to the entire weight of the larva, like the figures of Straus (*43*) on the percentage composition of the larva (fig. 15). The percentage of dextrose in the culture media gives the actual effective concentration of the sugar in the medium by weight. Since the unassimilated sugar is contained almost entirely in the intestine from which it is absorbed, the true concentration of sugar in the intestine should be determined in relation to the weight of the intestinal content. Furthermore, as suggested by Maassen (*28*), growth of *Bacillus larvae* occurs only inside the intestine until after the histolysis has begun, making possible the invasion of the body tissues by the organisms. It is therefore in the intestinal contents during the last part of the feeding period that the presence of sugar is primarily effective in inhibiting the growth of the organisms. The actual concentration of sugar in the intestine is, however, difficult to determine accurately, since the actual weight of food consumed by the larva for each 24 hours of the feeding period is unknown. Furthermore, the weight of the intestinal content is difficult to determine, because of the difficulty of dissecting the intestine free from the surrounding body tissues or of removing the contents intact.

Several attempts were made, however, to remove intestines with as little adhering tissue as possible from larvæ of different sizes during the last two days prior to sealing, in order to obtain an approximately accurate figure for the relation between the weight of the intestine and the weight of the larva. This in the several larvæ dissected was found to be almost always about 1 to 5. Using this factor,

the percentage concentration in the intestine, at least during the progressive feeding period, should be approximately five times as great as the value calculated (in Table V) on the basis of the entire larval weight. The calculated percentages for the third and fourth days are now 4.90 and 14.10, respectively, and on the fifth day, just before sealing, the sugar concentration in the intestine should approximate 15.70 per cent (fig. 16). There is, of course, the factor of dilution,

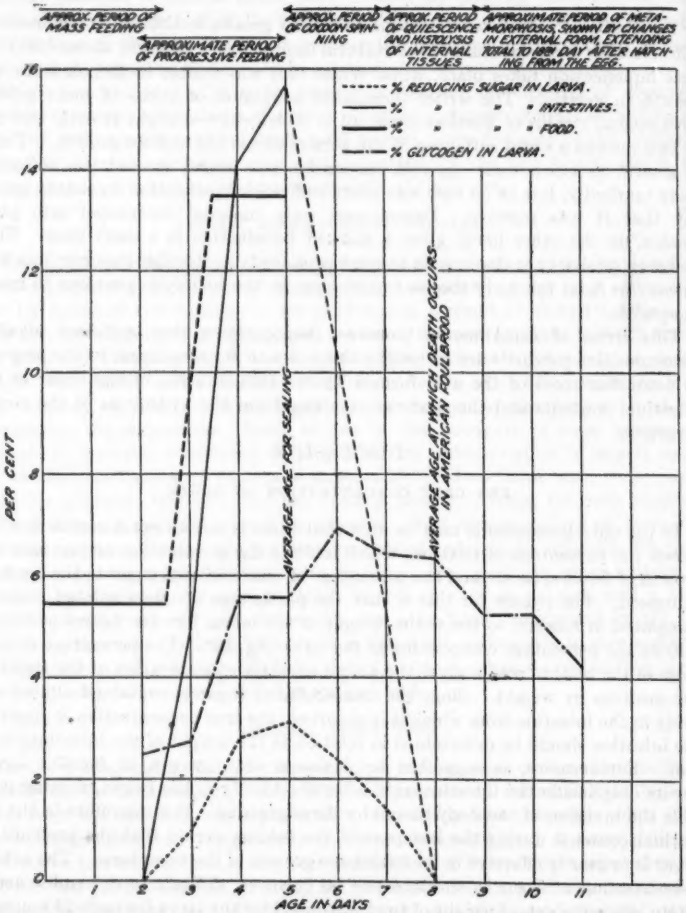

FIG. 16.—Correlation of time of death from American foulbrood to per cent concentration of reducing sugar in entire larva and to calculated per cent concentration in the intestine. Per cent sugar in the food and per cent glycogen in the larva are shown for comparison

particularly toward the end of the feeding period, caused by the accumulation of undigested pollen shells, which may lower this figure somewhat.

Still another approximate check may be calculated from the molecular weights of dextrose, glycogen, and fat, and from the percentage composition of the constituents of the larva (Tables IV and V), in order to obtain the percentage of sugar which was present in the intestine at any one time previous to assimilation neces-

sary for the formation of the stored glycogen and fat, on the basis of the relation of their carbon atoms.

	Formula	Molecular weight	Equivalent sugar per cent	
Dextrose (unassimilated)	$C_6H_{12}O_6$	180		[5]3. 14
Glycogen	$C_6H_{10}O_5$	162	$\frac{180-162}{180}\times$[5]5. 57 + 5. 57 =	6. 13
Fat (oleic acid)	$C_{18}H_{34}O_2$	282	$\frac{3\times180-282}{3\times180}\times$[6]3. 64 + 3. 64 =	5. 38
Total				14. 65

Since more sugar is used for energy in the production of a molecule of glycogen or of a molecule of fat (*32, p. 77*) than is indicated by the actual relation of the carbon atoms, this figure should be somewhat higher, thereby more nearly corresponding with the figure calculated from the weight ratio between intestine and larva.

The average concentration of sugar in the food of a larva of the age during which inhibition of bacterial growth takes place is 13.48 per cent (*40*) (Table III, fig. 11). The percentage composition of the larva at the different age periods (Table IV, fig. 15) in relation to food composition indicates, however, that the food probably is not assimilated as rapidly as it is ingested by the larva. If this is the case, an increase in the unassimilated sugar in the intestine would occur, as is indicated by the results obtained and the calculated percentage figures. The percentage of glycogen and fat increases slightly by the third day, as a result of the change in the composition of the food and the resulting increase in nursing (*26*). This is accompanied by the appearance of unassimilated sugar in the larva (Table V, fig. 15). There is then a marked increase by the fourth day, when apparently the limit for assimilation is reached, as shown by the constant percentage of glycogen in the larva as a whole between the fourth and fifth days in spite of the increase in weight, until after feeding ceases, when there is another increase in storage during the next 24 hours (sixth day). The latter is the result of the consumption of the food remaining in the cell after capping. The amount of unassimilated sugar increases continually, however, until feeding ceases, soon after the larva is sealed in the cell. This fact probably accounts for the slight difference between the percentage of sugar in the food and the calculated percentage in the intestine, but the correspondence is so striking as to substantiate the assumption.

Even though these calculations are only approximately accurate, it is known that some such condition must exist, since observations which have been made on the nursing habits of the honeybee (*26*), considered in relation to the figures for unassimilated sugar and food composition, give adequate foundation to the conclusion that there is considerably more than enough sugar in the intestine at the time infection occurs, or soon after, to inhibit the growth of the organism causing American foulbrood.

THE ACTIVE FEEDING STAGE IN THE LIFE HISTORY OF THE LARVA

The feeding stage of the honeybee larva has been divided into two parts, described by Lineburg (*26*) as the mass feeding period and the progressive feeding period. These two periods are characterized by a difference in the manner of feeding and in the amount of time spent by the nurse bees in the process, as well as by the change in chemical composition and character of the food (*40*) (Table III), and by the chemical composition of the larvæ themselves (*43*) (Table IV). It has been determined, however, that this change in composition of food occurs

[5] From Table V.
[6] From Table IV, Straus.

much earlier than stated by Von Planta (*35*, *26*). The young larvæ receive a food rich in nitrogenous material and relatively low in sugar and in which pollen grains are absent during about the first two and a half days of larval life. A large part of this, which is several times in excess of the weight of the larva during the first 24 hours or more (*35*) (Table VIII), seems to be placed in the cell with the newly hatched larva at one time soon after hatching, which justifies the assumption of mass feeding. During this period, assimilation must be very rapid, because the greatest relative growth occurs during the first two to three days (Table VIII) and also because no unassimilated sugar can be demonstrated in the larva during this period, even though the food contains about 5 per cent reducing sugar (Table V). The high nitrogenous content of the food apparently serves for rapid cell building, while the sugar is largely consumed in producing energy for this rapid growth; little storage of glycogen or fat occurs during this period. The nature and composition, as well as the biochemical reactions of this early food, as described by Koehler (*25*), suggest that it is a glandular secretion rather than a regurgitation of predigested honey and pollen from the ventriculus. The chances of larvæ of this age during mass feeding receiving infective material are, therefore, slight.

TABLE VIII.—*Ratio of weight in milligrams of unconsumed food in cell to weight of larva at different approximate ages (from Sturtevant (35))*

Approximate age (days)	Number of observations	Average weight of food per cell	Average weight of larvæ	Average ratio, food to larva
		Milligrams	*Milligrams*	
Egg	Several.	None.	[a] 0.10	0
1.1	33	3.96	1.02	3.88
1.4	131	3.23	1.36	2.37
2.1	24	9.10	7.20	1.26
2.6	35	11.79	17.48	.67
3.0	25	5.05	25.22	.20
3.5	65	7.75	63.46	.12
4.4	17	8.76	115.15	.08

[a] From Nelson (*35*).

Soon after the second day, a change in the composition of the food of the larva occurs, accompanied by a change in the method of feeding it by the nurse bees (*26*). The larva is now fed at approximately the rate at which the food is ingested by it, the demand for food rapidly increasing, accompanied by the great increase in actual body weight, until the time of sealing. The food now contains many entire pollen grains and has a much higher sugar content, nearly 14 per cent, and a relatively lower nitrogenous content (Table III). The principal ingredients are now honey or nectar and pollen. It is well known that honey which is gathered while disease is present in the hive usually carries infection. There is, therefore, a much greater opportunity for infection to occur when the larvæ are being given a food containing unmodified honey as one of its chief ingredients. Furthermore, the constant care of the larvæ during the period of progressive feeding and the large number of nurse bees which visit the cell still further increase the chances of infection being introduced during the period of progressive feeding. It is the young bees in the colony which act as nurses, and these bees are also the ones which clean the hive, so that they are more apt to have infected material on their mouth parts and elsewhere than are old field workers. There can, therefore, be little doubt that it is almost exclusively during the period of progressive feeding that infection normally occurs. Under normal feeding conditions the disease organisms can not develop in the larval intestine until after

feeding has ceased and the sugar-containing food has largely been assimilated, because, with the rate at which the larva is given food containing about 14 per cent sugar, the sugar concentration in the intestine must rapidly increase beyond the 3 to 4 per cent concentration which inhibits growth, so as to prevent the development of the disease in the larva. From Tables IX and X it may be seen that from the second day on, increasing numbers of visits are made to the larva with an increasing amount of time spent in nursing (Table X), so that on the last day of feeding a nursing visit, averaging six seconds in duration, is made approximately every 30 seconds, or an average total during the last 24 hours of 2,855 visits, or 36 per cent of the average 7,858 visits made during the entire feeding period (Table IX). The rapid consumption of this great amount of food, which is supplied in almost a steady stream, is indicated by the fact that the amount of food in the cell with the larva decreases to less than 10 per cent of the weight of the larva just prior to sealing. Even though the exact amount of food consumed may not be known, the actual concentration of sugar in the intestine during this period must rapidly rise, because of the high concentration of sugar in the food and the volume at which it apparently is fed to the larva, to several times the concentration necessary to inhibit the growth of *Bacillus larvae*. There is, as mentioned above, a diluting factor due to the accumulation of undigestible material until the connection is made with the end intestine, but, as shown by the microscopical examination of the intestinal contents, this, because it is largely insoluble, is probably of relatively slight importance in its effect upon the actual sugar concentration in the intestine during the period when inhibition occurs.

TABLE IX.—*Relation of nursing to increase in weight of larva* [a]

Age (days)	Average weight of larvæ	Average daily increase	Average daily ratio of increase to preceding weight	During active feeding period	
				Number of visits in 24 hours	Average increase in weight per visit per 24 hours
	Gram	*Gram*			*Gram*
Egg	0.000100				
1	.000650	0.000550	5.5	921.6	0.000000597
2	.004745	.004095	6.3	833.8	.00000492
3	.024626	.019881	4.19	1163.5	.00001709
4	.093990	.069364	2.82	2083.7	.00003329
5	.146748	.052758	.56	2855.5	.00001848
6	.155005	.008257	.06		

[a] From Sturtevant (*35*) and Lineburg (*16*).

TABLE X.—*Time spent in feeding* [a]

Age (days)	Average time spent in nursing per visit per 10 minute period	Average number of visits per 10 minute period	Average number of seconds per visit	Average frequency of visits	Average number of seconds between visits
	Seconds		*Seconds*	*Seconds*	*Seconds*
1	20.73	6.40	3.2	93.7	90.5
2	5.93	5.79	1.0	103.6	102.6
3	11.42	8.08	1.4	74.3	72.9
4	41.00	14.47	2.8	41.5	38.7
5	118.08	19.83	5.9	30.3	24.4

[a] Calculated from Lineburg (*16*).

Since it is shown that the concentration of reducing sugars in the larval intestine is usually sufficient to inhibit the growth of *Bacillus larvae* and thus to prevent the manifestation of American foulbrood until after sealing, it is now necessary to explain the rare cases in which advanced stages of the disease are seen in younger coiled larvæ. Such cases are exceedingly rare, except in colonies where almost every cell in the brood combs is filled with a dried scale, and where the bees have deserted the brood-nest because of this diseased material. There can be no doubt that in these cases the earlier manifestation of the disease is due to the fact that in such colonies the progressive feeding of the larvæ is seriously reduced by the fact that the colony has already been depleted in numbers of adult bees. Since there must be a decrease in progressive feeding in such cases, the concentration of reducing sugars in the intestine of the larva is obviously reduced, causing a condition to exist in these intestinal tracts which no longer inhibits the germination and growth of the causative organism. Such rare cases of young larvæ dead of American foulbrood do not, therefore, disprove the theory regarding the time of the development of the disease which has been here set forth, but rather serve as further substantiation of it.

THE COCOON-SPINNING STAGE

Sealing usually takes place on about the fifth day, at which time apparently the intestine contains a maximum amount of unassimilated sugar. After sealing occurs and feeding ceases, a different set of factors influence the concentration of sugar in the intestinal contents, so that there is a rapid steady decrease from this time on. The storage of glycogen and fat, however, continues for a short while from the assimilation of reducing sugar. Soon the movements of cocoon spinning and the histolysis of tissues make necessary the utilization of energy stored in form of glycogen and fat, so that the percentage of these substances begins to decrease as the larva loses weight. The emptying of the intestine of fecal material during this period also tends to decrease the sugar in the intestine, so that by the time the cocoon is finished, some time between the seventh and eighth days, the intestine is empty. The larva has straightened out and become quiescent by the eighth day, and all remaining sugar has now been assimilated. It is during this period that, as the concentration of sugar decreases, a point is reached where the growth of the organism can proceed. This apparently occurs when the sugar concentration in the intestine has decreased to about 3 to 4 per cent or less, probably not until some time between the sixth and seventh days. In the cultural experiments it was found that with 2 per cent or less dextrose, vigorous vegetative growth occurs. This vigorous growth requires, however, from 24 to 48 hours to develop, depending somewhat on the amount of initial inoculum. It is probable, as suggested by observations of Berman and Rettger (*6*), that this sugar in the food furnishes the energy for vegetative growth, while the soluble nitrogenous constituents of the intestinal contents furnish material for cell metabolism, until the time when the organisms have increased in number sufficiently to cause death and are able to invade the tissues of the larva, causing their subsequent decomposition.

THE QUIESCENT STAGE

As also is known from long observations of symptoms, the death of the larva and invasion of the body tissues do not in the majority of cases take place until after the larva has at least reached the age of 8 days and has become quiescent. This fact explains the characteristic uniformity of position and appearance of the majority of the larvæ dead from American foulbrood (figs. 7, 8, and 9). There are occasional cases in which death is still further delayed for some reason

until external transformation in form has begun, so that after death the pupa tongue is seen extended and often attached to the upper side wall of the cell in a characteristic manner (fig. 17.) It is possible that in such cases the initial inoculum was smaller than the average, thereby retarding maximum growth of the organism, as was noted in the cultural experiments, and delaying the production of sufficient toxin to kill until this stage of development had been reached.

While the biochemical relations of the bee larva to the disease are seemingly quite adequate to explain the delay in the time when American foulbrood is manifest, there is one other consideration which should be mentioned. The ability of the larva to resist the invasion of the bacteria is a subject on which virtually nothing is known, yet there must be some such ability, as is suggested by the fact that a slight initial inoculum in a colony may not cause the disease to be manifest. At the time when the biochemical conditions are most favorable for the germination and growth of the invading organisms, the larva itself has reached that stage of its development when its internal structure is materially modified by histolysis in advance of pupation, and it must follow almost necessarily that its power of resistance is reduced. The extent to which this factor is involved is, for the time being, purely a matter of speculation.

FIG. 17.—Decomposed, dried down remains of pupa dead from American foulbrood, showing characteristic tongue attachment to upper wall of cell (White (55)

The variation in the content of reducing sugar of the healthy honeybee larva and the inhibition of the germination and growth of *Bacillus larvae* by a concentration of over 3 or 4 per cent yield an interesting fact concerning the other serious disease of the brood of bees. European foulbrood makes its attack on the bee larva at an earlier stage in its development, while the content of reducing sugars is still high. It must, therefore, be concluded that *Bacillus pluton* has the ability to grow and rapidly to produce toxic substances sufficient to kill the larva in a medium of much higher reducing-sugar content than has *Bacillus larvae*, probably as high as 15 per cent.

THE EFFECTS OF BACTERIAL METABOLISM IN THE LARVA

There is little unanimity of opinion concerning the effect of dextrose (glucose) upon nitrogen metabolism by various bacteria. Kendall and Walker (*24*) concluded that the presence of glucose in the medium delays the production of proteolytic enzym, indicating the "protein-sparing" action of carbohydrates. Fischer (*23*) believed that proteolytic enzym is inactivated by glucose, indicated by inhibition of indol formation. Berman and Rettger (*6*) state that the presence of a carbohydrate in a culture medium may inhibit protein metabolism, depending on the nature of the medium and on the type of the organism as related to hydrogen-ion concentration. DeBord (*20*) believes that some bacteria destroy glucose without marked increase in the hydrogen-ion concentration and that the rate of production of amino nitrogen or ammonia nitrogen, which may be affected by the presence of carbohydrates, indicates different types of metabolism of bacteria.

The results of the present investigation, although more or less incomplete on this subject, seem to indicate that *Bacillus larvae* has the ability to decompose nitrogenous material in the presence of carbohydrate, since there must be dextrose available until the stored glycogen of the fat body is entirely hydrolyzed.

Many organisms are unable to attack complex protein unless there is some other source of food present, because, according to Berman and Rettger (*6*), to be

utilizable by bacteria sufficient growth is necessary by which to produce the enzym capable of splitting the complex protein molecule into its simpler amino-acid forms. In the healthy larva at the age when *Bacillus larvae* starts growth in the intestine, the remaining sugar in the intestinal contents and other material of nitrogenous nature in the food is sufficient, as stated earlier, to produce the energy for the initial growth of the organism. By the time the invasion of the tissues by the organisms occurs sufficient proteolytic enzym has been produced to attack the body proteins. Furthermore, the process of histolysis itself, as stated in relation to gelatin liquefaction by ropy material, has probably broken up sufficient of the body proteins to serve as food for bacterial metabolism.

Since *Bacillus larvae* belongs to the spore-forming group of organisms, its processes of metabolism may be similar to those of *Bacillus subtilis*, as described by Berman and Rettger (*6*), as follows: "The ability of *Bacillus subtilis* to break down protein in the presence of fermentable sugar, and in the absence of an added buffer, may be explained as follows. This organism attacks glucose slowly, and for this reason it is able to produce its proteolytic enzym before the hydrogen-ion concentration reaches a point unfavorable to further growth. When the enzym is thus formed the products of the nitrogen metabolism neutralize the acid, at least in a measure, and the metabolism therefore continues uninterruptedly."

The fact that the production of indol and even of ammonia can be demonstrated, although they may be produced slowly and in small amounts, indicates that even though considerable acid may be produced by the fermentation of the carbohydrate in the food and the hydrolysis of the glycogen, *Bacillus larvae* has putrefactive functions which bring about the formation of sufficient alkaline protein-digestion products from the larval tissues to neutralize this acid production, thereby maintaining the hydrogen-ion concentration at approximately P_H=6.8.

SUMMARY AND CONCLUSIONS

1. It has been shown by the work of others that the glycogen and fat content of the bee larva increases in a definite manner for a time and then decreases. In the present work it is shown that the per cent and amount of reducing sugar likewise increase after the third day of larval feeding, but decrease rapidly immediately after feeding has ceased, until by the eighth day no reducing sugar remains in the larva.

2. The presence of reducing sugar can be determined only after the progressive feeding of the larva begins.

3. The best method for extracting reducing sugars from the bee larva was found to be by the use of 50 per cent alcohol and the removal by filtration of the insoluble materials. By this means the interference of glycogen with the action of the reducing solutions is prevented.

4. The constitution of the protein molecule of the normal tissue content of the healthy bee larva is complex, containing, however, among other amino-acids, tryptophan.

5. The best medium for the growth of *Bacillus larvae* so far devised is a yeast-extract agar medium to which sterile egg-yolk suspension has been added. The optimum reaction for cultural growth is P_H=6.8.

6. Reducing sugars in the culture medium of more than 3 or 4 per cent usually inhibit germination of spores and growth of *Bacillus larvae*. A few spores may germinate at higher concentrations, but the resulting vegetative forms fail to increase in number and show granular disintegration due to autolysis. Less than 2 per cent of reducing sugars seems to stimulate growth of the vegetative forms.

7. The food of the older honeybee larva contains a high percentage of reducing sugar, which is derived from the honey or nectar used in its production. The concentration of reducing sugar in the larval intestine is more than sufficient to inhibit the growth of *Bacillus larvae* until after feeding has ceased. After feeding ceases, the remaining reducing sugar is rapidly assimilated, so that by the seventh day the concentration of sugar has been reduced sufficiently for the active growth of *Bacillus larvae* to occur.

8. The incubation period of *Bacillus larvae* is 24 to 48 hours, so that growth sufficient to kill the larva does not occur until it has completed the spinning of its cocoon and has extended quiescent in the cell, on or after the eighth day, by which time all reducing sugar has disappeared from the larva.

9. The delayed death of the larva in American foulbrood is, therefore, correlated with the inhibiting effect of unassimilated reducing sugar in the intestine upon the germination and growth of *Bacillus larvae*.

10. *Bacillus larvae* has the ability to produce considerable acid, but the hydrogen-ion concentration of the decomposing material is not thereby increased, because of the neutralizing effect of protein decomposition products. The hydrogen-ion concentration of the diseased larva throughout its decay varies only slightly from $P_H = 6.8$.

11. *Bacillus larvae* not only utilizes reducing sugar for its initial growth, but also completely hydrolyzes the glycogen of the larval body tissues in the process of decomposition.

12. *Bacillus larvae* has the ability to decompose nitrogeneous materials, with the formation of amino-acids, indol, and ammonia, but the hydrogen-ion concentration is not decreased by this action, because of the concomitant production of acids from carbohydrates.

13. *Bacillus larvae* apparently has no action on fat.

14. The biochemical data herein presented for the first time explain the remarkable characteristics of American foulbrood, which were left entirely unexplained from observations on etiology alone.

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BRITTLE STRAW AND OTHER ABNORMALITIES IN RYE[1]

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In America rye is of minor importance as a grain crop; consequently there has been relatively little time devoted to its improvement. However, rye combines many desirable qualities such as winter hardiness, wide soil adaptation, quick growth, and good yield. It seems probable, therefore, that it will eventually find wider favor in this country.

Two general methods of improvement of rye are employed at the Minnesota Agricultural Experiment Station, namely, bulk selection for purity of seed-color and for vigor of the plants, and selection in self-fertilized lines. Minnesota No. 2 rye, which originated at Minnesota as a result of selection for winter hardy plants in Swedish rye, has been used for all of the inbreeding work. Since rye is normally cross-fertilized, commercial varieties are ordinarily heterogeneous mixtures for seed color and plant type. Both of these methods of breeding seek the isolation of vigorous uniform types, and hence may be considered respectively as broad and narrow types of inbreeding.

One of the interesting features of these studies at Minnesota is the occurrence of certain types of abnormalities, in inbred strains. Since the normal type is usually dominant when in the heterozygous condition such abnormalities are seen only rarely in a commercial variety. Inbreeding, either by selection or by self-fertilization, brings about a recombination of the recessive abnormality factors and results in the appearance of abnormal plants in the inbred strains. This is of the utmost importance since it gives the plant breeder an opportunity to isolate vigorous desirable types and to discard the undesirable ones. It is the purpose of this paper to present some of the data at hand regarding these abnormalities, with particular reference to one called "brittle" on account of the ease with which the straw breaks.

Chlorophyll deficiency is of common occurrence as an abnormality in inbred strains of rye. In 1922, white or "albino" seedlings were found in nearly 25 per cent of the self-fertilized strains and in more than 50 per cent of the strains originated by bulk selection, as shown in Table I.

TABLE I.—*Occurrence of chlorophyll-deficient abnormalities in inbred strains and in normal Minnesota No. 2 rye, in 1922*

	Strains isolated by bulk selection	Self-fertilized strains	Normal Minnesota No. 2 rye
Number of strains in the rye nursery	18	200	.....
Number of strains segregating for white seedlings	13	45	.....
Range of percentage of white seedlings to normal green seedlings in the inbred strains	0.4-32.4	4-60	0.04
Number of strains segregating for other types of chlorophyll abnormalities	3	23	.....

[1] Received for publication Nov. 22, 1923.

TABLE II.—*Occurrence of "brittle" plants in selfed-strains of rye, in 1922*

1922 culture number	Years selfed	Normal plants	Brittle plants
R 11	1, D–P [a]	28	2
R 19	1, D–P	17	5
R 36	1	6	3
R 37	1	5	1
R 39	D–P [b]	12	6
R 41	1	16	2
R 43	D–P	14	7
Total		98	26

[a] 1, D–P=one year selfed followed by D–P.
[b] D–P=intercross, that is, two heads on different plants in the same culture bagged together.

Chemical analysis of the plants was undertaken to determine the cause of brittleness. Samples of brittle rye and normal rye were put up while green in 85 per cent ethyl alcohol containing sufficient $Ca(CO_3)2$ to neutralize any acids.

TABLE III.—*Analysis of normal and brittle rye culms*

Sample	Dry weight	Per cent ash	Sugar			Pentosans	Starch	Total pectin	Crude (dry basis)	Fiber (wet basis)
			Red	Sucrose	Total					
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Brittle rye	27.03	5.49	7.09	6.47	13.57	34.34	0.78	0.234	13.50	3.81
Do		5.44	7.12	6.50	13.60	33.35	.81	.242	14.75	
Normal rye	40.58	4.91	5.60	4.63	10.26	25.40	.98	.20	32.21	13.04
Do		4.85	5.67	4.79	10.42	24.60	1.04	.20	32.10	

In Table III a comparison of the principal chemical constituents which may be associated with the production of brittle or healthy plants is given. The percentage of dry weight of the brittle rye and of the normal rye shows quite an appreciable difference. The brittle rye contains the lower percentage of dry material. This suggests a high content of colloid with high water-holding capacity, a condition which is indicated by the greater pentosan content of the brittle plants. Culms that had a high water content or were turgid would have a tendency to snap off easily as the brittle plants do.

The crude fiber determination shows the relative amount of skeletal material of the plants, particularly cellulose and lignin, which are responsible for the strength of the culm. There is a ratio of 32.15 per cent crude fiber in the normal plants to 14.13 per cent in the brittle plants, comparison being made on the dry weight basis. When calculated on the wet weight basis, the ratio of crude fiber of the normal rye to the brittle is 13.04 to 3.81 per cent. The proportion of crude fiber on the wet weight basis is the proportion we would find in the stalk in the field, and this extreme difference in crude fiber may explain to a great extent the difference in the strength of the stems.

The pentosan content of the brittle rye is higher than that of the normal rye on the dry weight basis, but is almost the same on the wet weight basis. The pentosan material being chiefly hydrophilic colloids does not aid appreciably in the skeletal make-up or strength of the stalk, except as it enters into the composition of the ligno celluloses. This higher percentage of pentosan in the brittle rye may explain the higher water content of the brittle stalks, when compared with the normal ones.

The ash, starch, and pectin contents are not very different in the two samples. The high sugar content and higher pentosan content coincident with low crude fiber in the brittle plants indicate that the carbohydrates do not contribute to wall-forming material in the normal way.

The methods used in the analysis of ash, starch, crude fiber, and pentosan were taken from the "Methods of Analysis of the Association of Official Agricultural Chemists." [2] The method used for the pectin determination was that

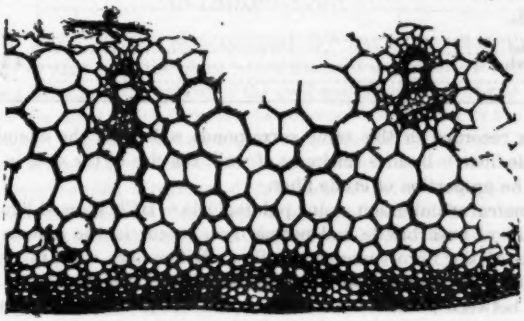

FIG. 1.—Cross section of normal rye stem. ×80

reported by Carré and Haynes.[3] The sugars were determined by the picric acid method.[4]

Histological and microchemical examinations were made along with the chemical analysis. The microscopic appearance of cross sections of brittle and normal rye stems was quite different, as is shown in figures 1 and 2. While the depth of the sclerenchyma layer at the periphery of the stem was practically the same in the two samples, the thickness of the cell walls was strikingly less

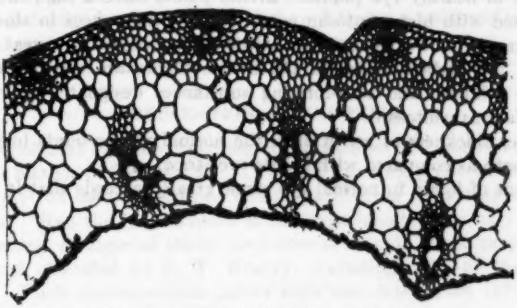

FIG. 2.—Cross section of "brittle" rye stem. ×80

in the brittle rye, as indicated in Table IV. Garber and Olsen found a similar condition associated with lodging in oats and rye.[5]

[2] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. As compiled by the Committee on Revision of Methods. Revised to Nov. 1, 1919. 417 p., illus. Washington, D. C. 1920.

[3] CARRÉ, M. H., and HAYNES, D. THE ESTIMATION OF PECTIN AS CALCIUM PECTATE AND THE APPLICATION OF THIS METHOD TO THE DETERMINATION OF THE SOLUBLE PECTIN IN APPLES. Biochem. Jour. 16: 60–69. 1922.

[4] LEWIS, R. C., and BENEDICT, S. R. A METHOD FOR THE ESTIMATION OF SUGAR IN SMALL QUANTITIES OF BLOOD. Jour. Biol. Chem. 20: 61–72. 1915. (Modified 1923 by Willaman and Davison.)

[5] GARBER, R. J., and OLSEN, P. J. A STUDY OF THE RELATION OF SOME MORPHOLOGICAL CHARACTERS TO LODGING IN CEREALS. Jour. Amer. Soc. Agron. 11: 173–186, illus. 1919.

TABLE IV.—*Thickness of the cell wall in brittle and normal rye*

	Brittle rye	Normal rye
	Microns	*Microns*
Epidermis:		
External wall	2.4-2.9	5.3
Side wall	.9	3.3
Sclerenchyma	2.8	9.6
Vascular bundle:		
Sheath	.4-1.9	4.8
Pitted vessel	1.4	2.4
Spiral vessel	3.2-4.8	4.8
Pith cell	.4- .9	2.4

The data recorded in this table corresponds well with the chemical estimation of crude fiber. Besides thickness of wall, the size of the cells is a factor determining the proportion of crude fiber.

Microchemical examination, using phloroglucin —HCl, showed lignin throughout the walls of both brittle and normal rye, except in the phloem and xylem parenchyma of the fibro-vascular bundles. Differences were in degree rather than in extent of lignification. It was not found practicable to attempt to distinguish between pentosans in the wall and the protoplast, because pentoses demonstrated in the vacuole could easily have diffused in from the hydrolized pentosans of the wall.

SUMMARY

Brittleness of straw, chlorophyll deficiency, male sterility, and crinkled awns were found as abnormalities in inbred strains of Minnesota No. 2 rye.

Chemical analyses show a low percentage of crude fiber (14 per cent) and high pentosan (34 per cent) content in brittle straw compared with a crude fiber content of 32 per cent and pentosan content of 25 per cent on a dry-weight basis in healthy rye plants. Brittle plants have a high moisture content correlated with high pentosan content. The differences in starch, pectin, ash, and sugar content of normal and brittle rye plants are not great in amount.

Evidently the carbohydrates in the brittle plants are not normally transformed into cellulose and wall-forming substances designated as crude fiber, but accumulate as pentosans.

The greater thickness of cell wall in the normal rye accounts in part for its greater strength as compared with brittle rye straw.

The amount of lignin in normal rye straw greatly exceeds that in brittle rye

RELATION BETWEEN TOXICITY OF COTTONSEED AND ITS GOSSYPOL CONTENT [1]

By ERICH W. SCHWARTZE, *Pharmacologist in Charge, Pharmacological Laboratory,* and CARL L. ALSBERG, *formerly Chief, Bureau of Chemistry, United States Department of Agriculture*

INTRODUCTION

Analysis of many samples of cottonseed (*9*)[2] has shown that the gossypol content tends to vary with the place where the seed is grown. A positive correlation between the gossypol content of the seed and its oil content was also found. The fact that the seeds which were low in gossypol were grown in the Southwest where cottonseed poisoning is uncommon supports the hypothesis that the tendency toward a regional variation of the gossypol content of the seed accounts for the tendency toward a regional distribution of cottonseed poisoning. The experiments herein reported deal with this hypothesis. The toxicity of four samples of raw cottonseed kernels having extremely high and moderately high gossypol contents, as observed in animal experiments, is compared with their toxicity as estimated from their gossypol contents. It was thought that if the toxicities in each case should prove to be similar or identical, such parallelism would go far to indicate that gossypol is the cause of cottonseed poisoning (*1, 2*).

EXPERIMENTAL MATERIAL AND METHODS

KERNELS (MEATS) AS THE SOURCE OF POISON

The kernels (meats), practically free from hulls and lint, rather than the commercial meal, were used in order to eliminate any effect upon the toxicity of the seed resulting from the heat and pressure employed in the process of expressing oil. Moreover, the method of estimating gossypol in cottonseed press cake has not been sufficiently developed. It is believed that a considerable quantity of the decomposition products of gossypol is present in the meal. Concerning their pharmacological action little is known.

PREPARATION OF GOSSYPOL

The gossypol used in these experiments was isolated and purified as the "acetate" by the method of Carruth (*3*). Two large lots were prepared from different samples of gin-run seed and a third was prepared from a mixture of select and gin-run seed. Had the requirements of the experiments been fully anticipated in the beginning, a single lot would have been prepared. Each preparation was examined and identified by E. T. Wherry, crystallographer of the Bureau of Chemistry. Their decomposition points were also determined (176°–180° C.). The preparations fed were freed from the acetic acid by washing an ether solution of gossypol acetate three times with water.[3] The ether solution was poured into a dish of water and evaporated on the steam bath. The crusts of gossypol were dried in the air and later ground.

[1] Received for publication, Mar. 6, 1924.

[2] Reference is made by number (italic) to "Literature cited," pp. 188–189.

[3] Gossypol "acetate" seems to be unstable in ether solution. The precautions to free the gossypol from acetic acid may have been superfluous, as hydrolysis may occur also in other solvents, such as fat or oil.

PREPARATION OF EXTRACTS OF COTTONSEED KERNELS

The cottonseed kernels were extracted in a Soxhlet extraction apparatus until no significant yellow color was imparted to the ether which bathed the thimble and contents, a procedure that required from 18 to 48 hours. The ether was evaporated from the extract with the aid of heat, and the residual oil, diluted with peanut oil, was used for the experiments in the manner described.

DIETS

In the feeding tests four diets were used: Control peanut meal; gossypol; raw cottonseed kernel; and control ether-extracted cottonseed kernel. The first three contained an abundance of peanut meal, an adequate source of protein. All diets contained an abundance of butterfat as a source of fat-soluble vitamin, an abundance of milk powder supplying protein, and an adequate salt mixture. They contained roughage in the form of agar-agar, except those containing the greatest quantity of each variety of raw cottonseed kernels, which were used only for a few of the shorter feeding experiments.

The control peanut meal diet and the gossypol diet differed only in the presence of gossypol. The control ether-extracted cottonseed kernel diet was the same as the other diets, except that it contained ether-extracted cottonseed kernels and additional fat instead of peanut meal or whole cottonseed kernels. The gossypol and fat had been removed from the cottonseed kernels by ether extraction in the course of quantitative gossypol analyses formerly made (*9*). The raw cottonseed kernel diet was the same as the control diet except that part of the peanut meal and fat had been replaced by different varieties of raw cottonseed kernels in varying proportions. The composition of the various kernels and diets is given in Tables II and III. In each case the diet contains all the ingredients essential for normal growth.

TABLE I.—*Ether extracts injected intraperitoneally*

Variety extracted	Cottonseed		Ether extract		Gossypol in diluted extract for injection
	Quantity extracted	Gossypol content	Gossypol content	Peanut oil added to make—	
	Gm.	*Per cent*	*Mgm.*	*Cc.*	*Per cent*
Trice	54.6	0.411	224	50	0.449
Lone Star	42.2	.518	218	50	.437
Durango	26.2	.984	257	50	.514
Egyptian	19.2	1.180	227	50	.454

TABLE II.—*Composition of cottonseed kernels and other material*

Material	Ether extract	Nitrogen	Moisture	Gossypol
	Per cent	*Per cent*	*Per cent*	*Per cent*
Trice kernels	28.87	6.42	6.12	0.416
Lone Star kernels	33.40	6.40		.518
Durango kernels	38.97	4.94	5.93	.984
Egyptian kernels	36.68	4.73	5.66	1.180
Ether-extracted cottonseed kernels I [a]	[b] 0	9.01	10.92	[b] 0
Ether-extracted cottonseed kernels II [a]	[b] 0	8.59		[b] 0
Peanut meal I	(c)	8.30		0
Peanut meal II	10.33	8.11	6.59	0

[a] Composite samples resulting from the chemical analyses reported by Schwartze and Alsberg (*9*).
[b] Gossypol and fat were removed by ether extraction. A trace of each was probably present.
[c] Only a few experiments were made with this sample. Ether extract was assumed to be 10.33 per cent in making the calculations.

TABLE III.—*Composition of diets*

Diet	Ingredients						Composition		
	Cotton-seed kernels	Peanut meal	Ether-extracted cotton-seed kernels	Butter-fat	Refined oil [a]	Milk powder	Gossy-pol	Pro-tein [b]	Fat [b]
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Peanut I [c]		40.0		25.0		20.0	0.0	22.8	34.6
Peanut II [c]		40.0		25.0		20.0	.0	23.3	34.6
Ether-extracted cottonseed kernels Ia [c]			35.0	30.0		20.0	.0	21.0	35.5
Ether-extracted cottonseed kernels Ib [c]			38.0	25.0	2.0	20.0	.0	22.0	32.5
Ether-extracted cottonseed kernels II [c]			33.6	17.0	14.4	20.0	.0	20.0	36.9
Gossypol (0.0675) [c]		40.0		25.0		20.0	.065	23.3 to 22.8	34.6
Gossypol (0.1350) [c]		40.0		25.0		20.0	.135		
Gossypol (0.2250) [c]		40.0		25.0		20.0	.225		
Trice (15) [c]	15.0	30.0		20.0		20.0	.062	24.0	32.9
Lone Star (13) [c]	13.0	31.0		21.0		20.0	.067	23.7	34.0
Durango (7.5) [c]	7.5	36.5		21.0		20.0	.074	23.8	33.2
Egyptian (6) [c]	6.0	37.0		22.0		20.0	.071	23.4	33.5
Trice (30) [c]	30.0	20.0		15.0		20.0	.123	24.7	31.2
Lone Star (26) [c]	26.0	20.0		19.0		20.0	.135	23.3	35.3
Durango (15) [c]	15.0	30.0		20.0		20.0	.148	22.8	34.5
Egyptian (12) [c]	12.0	32.0		21.0		20.0	.142	22.7	34.2
Trice (54.6) [d]	54.6	6.7		13.7		20.0	.224	27.3	35.7
Lone Star (42.2) [e]	42.2	18.9		13.7	.2	20.0	.218	28.5	37.8
Durango (26.2) [e]	26.2	31.3		13.7	3.8	20.0	.257	26.4	36.5
Egyptian (19.2) [e]	19.2	36.5		13.7	5.6	20.0	.227	26.7	35.6

[a] Refined but not deodorized cottonseed oil made in the Oil, Fat, and Wax Laboratory of the Bureau of Chemistry.

[b] The factor 5.5, used for calculating the protein of peanut meal and of cottonseed, is sufficiently accurate for these experiments. Commercial samples of whole milk powder have been assumed to contain approximately 25 per cent of protein and 27.5 per cent of fat.

[c] Corn starch (9 per cent), agar-agar (3 per cent), and salt mixture IV (3 per cent) (Osborne and Mendel, 7, *p.* 374) were supplied in these diets.

[d] This diet was not used, as the supply of seed became exhausted.

[e] These diets contained 2 per cent of corn starch and 3 per cent of salt mixture IV. No agar-agar was added.

Since gossypol is not evenly distributed in the seeds, but is found in the "gland dots" of the kernels (*10*), it seemed advisable to subject the kernels intended for feeding to a treatment which would uniformly distribute the gossypol through them. Accordingly, the kernels were ground and mixed with peanut meal. The mixture of peanut meal and finely ground kernels was then treated with ether. After having been warmed sufficiently, with constant stirring, the ether was evaporated on the steam bath, and the resulting dry lumpy meal was sifted and exposed for 18 hours to the atmosphere in thin layers on a dry glass plate. No odor of ether was apparent, and the food made from the meal was as acceptable to animals as that containing comparable quantities of gossypol.

METHODS OF ADMINISTRATION

Methods of administering the cottonseed kernels have been described. In the experiments in which free gossypol was added to the diet, it was incorporated in the butter fat used in the preparation of the diet. Possibly the gossypol entered into combination with some one of the substances present in butter, for in the more concentrated butter-fat solutions used in another series of experiments the gossypol crystallized out. It is not known whether or not gossypol is loosely combined with an organic acid in the cottonseed. In several feeding and intraperitoneal injection experiments, however, no difference in toxicity between gossypol and gossypol acetate was observed. It is therefore reasonable to con-

clude that any loose compound that may have been formed in butter would not materially influence the results of these experiments. Whatever the form in which the gossypol eventually may have been found and whatever its source, care was taken that it should be distributed evenly throughout the medium and that the state of subdivision should be as fine as the condition of the preparation would permit.

Whenever the gossypol was not administered *per os* it was given dissolved in peanut oil. Published data on the chemistry of gossypol, as well as the authors' experience, indicate that the best way to prepare this substance for intraperitoneal administration is to dissolve it in oil. The rate of oxidation as judged by color changes is much less in this medium than in aqueous alkali or in alcohol. Where the object was to determine the toxicity of a given sample of cottonseed rather than the toxicity of gossypol itself in the pure state, the ether extracts of the seeds to be tested were used. Their method of preparation has been described. It is obvious that for these experiments the proper procedure was to use the whole ether extract rather than pure gossypol prepared from a given sample of seed. The oil solution of gossypol and the oily residue obtained in the ether extraction of cottonseed kernels were always administered intraperitoneally, the most suitable way to inject an oil solution.

ANIMALS USED

Under the conditions obtaining in these experiments, rats appear somewhat unsusceptible to gossypol intoxication and do not show some of the typical symptoms of cottonseed poisoning found in certain farm animals. Because of the convenience of performing a large number of experiments simultaneously, however, they were used. The results establish the relation of the toxicity of different samples of seed to one another and to pure gossypol. All animals were of the same sturdy stock, reared in the laboratory. Variability was thus reduced to a minimum.

A few grown mice and a number of grown rats were used. In the early feeding experiments, however, growing rats proved best for the tests, so that the discussion is confined chiefly to experiments with them.

RESULTS OF EXPERIMENTS

INTRAPERITONEAL INJECTION OF PURE GOSSYPOL AND OF THE ETHER EXTRACT OF DIFFERENT VARIETIES OF COTTONSEED KERNELS

Gossypol was injected intraperitoneally because it is readily absorbed from an oil in the peritoneal cavity.[4] Gossypol is more potent by this route than by oral or subcutaneous administration. Apparently the injected gossypol leaves the oil and some of it is dissolved in serum exudate. The oral administration of gossypol dissolved in oil is followed by diarrhea and also by paralysis of the intestine and stomach musculature. From these phenomena it follows that the dosage varies with the degree to which the local phenomena are superimposed upon one another. In one case gossypol was recovered in the oil found in the stomach, the oil having been given by a stomach tube seven days before death. Absorption from the subcutaneous tissue is very poor. The intravenous administration of an emulsion of an oil solution of gossypol was not attempted because with the relatively small quantities of gossypol available no means were at hand for the preparation of an emulsion sufficiently fine to preclude fat embolism.

[4] Later experiments have shown that cottonseed oil becomes emulsified slowly, whereas peanut oil does not. No deleterious symptoms from the injection of pure cottonseed oil or peanut oil were observed during the lifetime of the animals.

The results of the intraperitoneal tests are given in Table IV. With a few exceptions, the percentage of gossypol dissolved in oil was 0.45 per cent. The minimum lethal dose is about 20 mgm. and the largest survived dose is about 50 mgm.[5] Smaller rats seem to require larger doses than larger animals. Individual differences in susceptibility to cottonseed poisoning between animals of the same age have been observed by other investigators. An effort was made to eliminate these as far as possible in the rat studies by using only animals that had been reared in the laboratory under standard conditions. Deaths from the smaller doses are delayed and to a great extent give the impression that the effect of starvation is superimposed upon an intoxication factor. Although 45 to 50 mgm. per kilo may be regarded as the practically certain fatal dose, all deaths did not occur within 24 hours as they did when larger doses were administered.

Tests with the ether extract of Egyptian cottonseed kernels indicate that these kernels are as toxic as the gossypol content determined by analysis would show them to be. The results in this case are even more uniform than those in the tests in which pure gossypol was used.

Tests with the ether extract of Durango cottonseed kernels also indicate that these kernels are as toxic as the gossypol content determined by analysis would show them to be. At the time these experiments were made, the gossypol content of the extract was underestimated by about 10 per cent. This explains why the dosage reported is slightly larger than in the case of the Egyptian seed extract.

TABLE IV.—*Toxicity to rats of gossypol and of volumetric solutions of ether extracts of cottonseed of which the gossypol content has been estimated (oil solution injected intraperitoneally)*

Number of rats	Weight	Dose of gossypol (calculated)	Fatalities							Survived	Remarks
			First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Total		
	Gm.	*Mgm. per Kilo*									
GOSSYPOL											
4	241-278	10-17	0	0	0	0	0	0	0	4	
1	244	18.5-20	0	1	0	0	0	0	1	1	
5♀	100-215	18	0	0	0	0	0	0	0	5	
4♀	177-205	18	0	0	3				3	1	All pregnant.
5♂	131-202	22.5	0	0	1	0	0	3	4	1	All refused food.
2	181-265	25-30	0	2	0	0	0	0	0	2	One pregnant.
5♂	125-175	31.5	0	2	3				5	0	
3	198-254	33-35	0	2	0	0	0	0	2	1	
3	70-80	36	0	0	0	0	0	0	0	3	Killed on 12th day.
5♂	146-177	45	2	2	1				5	0	
2	68-78	45	0	0	0	1	1		2	0	
2	159-187	45-49	1	1					2	0	
1	189	49	0	0	0	0	0	0	0	1	
7	64-195	55-90	7						7	0	
EGYPTIAN COTTONSEED											
5♂	135-165	18	0	0	0	0	0	0	0	5	One dead on 9th day.
5♂	145-177	45	5						5	0	4 died within 16 hours.

[5] Experience with this intraperitoneal fixed-oil method, using a large number of fat-soluble dyes, also reveals a variation in the toxicity of individual substances. This must be controlled by using a sufficient number of animals and making comparisons of the toxicity ranges obtained.

TABLE IV.—*Toxicity to rats of gossypol and of volumetric solutions of ether extracts of cottonseed of which the gossypol content has been estimated (oil solution injected intraperitoneally)*—Continued

DURANGO COTTONSEED

Number of rats	Weight	Dose of gossypol (calculated)	Fatalities							Survived	Remarks
			First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Total		
	Gm.	*Mgm. per Kilo*									
5♀	125–130	15.3	0	0	0	0	0	0	0	5	
5♂	145–175	20.7	1	0	0	0	0	0	1	4	
5♂	140–210	20.7	3	0	0	0	0	0	3	2	1 died on 12th day.
5♂	140–177	51.3	4	0	0	0	0	0	4	1	4 died within 16 hours.

LONE STAR COTTONSEED

5	112–222	17.5	0	0	0	0	0	0	0	5	All lived.
5♀	130–155	43.7	0	0	0	0	0	0	0	5	2 died later
5♀	210–260	65	3	0	1	0	0	0	4	1	

TRICE COTTONSEED

5♂	195–215	18	0	0	0	0	0	0	0	5	1 died on 15th day.
5♀	132–147	45	0	3	1	0	0	0	4	1	1 killed on 10th day.
5	127–145	58.5	3	0	0	0	0	0	3	2	2 killed on 10th day.

The tests with the ether extract of the Lone Star cottonseed kernels indicate that their toxicity is not so great as might have been inferred from their gossypol content as determined by analysis. The best estimate that can be made is that the seeds are only about two-thirds as toxic.

The tests made with the ether extract of the Trice cottonseed kernels indicate that the seed is slightly less toxic than indicated by the gossypol content as determined by analysis, the best estimate being about three-fourths of the theoretical value.

The Durango and Egyptian seeds which contained the larger quantities of gossypol proved as toxic as predicted on the basis of the chemical analysis, whereas Lone Star and Trice seeds were less toxic than predicted. Had the toxicity been underestimated instead of overestimated, evidence that some factor other than the gossypol was involved would have been furnished. This slight deficiency of toxicity, however, is not inconsistent with the gossypol hypothesis of cottonseed poisoning. This substance is not entirely stable, and a small quantity could easily have been oxidized after picking the seed. Nothing concerning the treatment, fumigation, or storage of these samples of seed was ascertained. Moreover, the extracts represent crude gossypol, and the method of analysis includes the estimation of a small quantity of some substance other than gossypol, presumably a decomposition product of gossypol.

A comparison of the results in Table I with those in Table IV shows that the order of toxicity of the seed used is the same as that indicated by the chemical analysis, but that the Egyptian seed, instead of being about three times as toxic as Trice cottonseed, is about four times as toxic. Analagous relations exist for Lone Star seed. These somewhat aberrant results may be within the possible range of error of the analytical method.

FEEDING EXPERIMENTS

CONTROL DIET OF PEANUT MEAL AND CONTROL DIET OF ETHER-EXTRACTED COTTONSEED KERNELS

The growth curves of the rats fed the control diets are given in figures 1 and 2. The charts have been compared with 50 gm. body weight as a base line or arbitrary starting point, since weight rather than age was the criterion of comparison. The average rate of growth for males and for females has also been

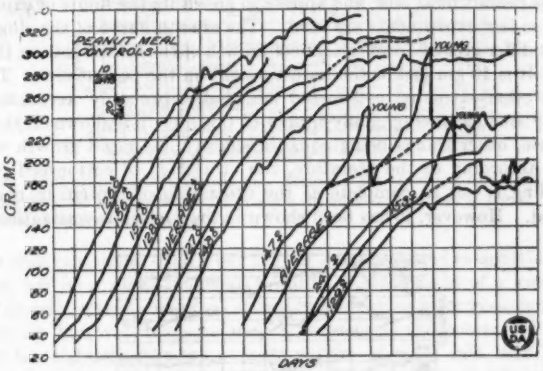

FIG. 1.—Growth of male and female rats on control peanut meal diet. Broken line on "average" curve represents estimated average rate of growth from fewer observations than for unbroken line. Peaks on female growth curves labeled "young" represent weight fluctuation due to pregnancy and birth of young. Broken line on curves of female rats represents hypothetical course of weight variation if pregnancy had not occurred

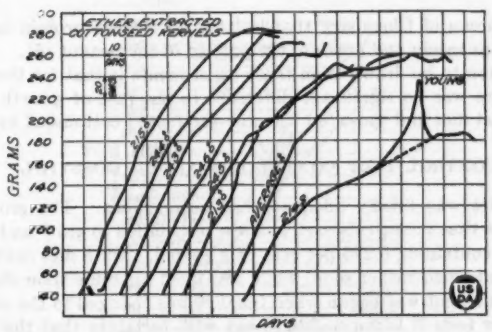

FIG. 2.—Growth of male and female rats on ether-extracted cottonseed kernel diet. No. 213, 214, and 215 were fed 30 per cent butter fat and the remainder 17 per cent butter fat. Broken line on one curve represents estimated weight if pregnancy had not occurred

computed and is shown on the charts. The rate of growth was much faster than that usually recorded in the nutrition literature and seemed to be associated with the sturdiness of the rats and with the high protein diet. The rapidity of growth necessitated the selection of 10 days for each unit on the abscissa instead of the conventional 20-day period, in order to keep the growth curves in the neighborhood of the 45-degree line.

Eighty days after the 50-gram body weight had been attained, the weight of the six male rats fed peanut meal ranged from 255 to 284 gm. with 273 gm. as an average. On the fortieth day the range for the four female rats was 147 to 174 gm., with an average of 157 gm.

The range of weights for the six males receiving the ether-extracted cottonseed kernel diet was 245 to 282 gm., with an average of 259 gm. on the eightieth day. The different butter fat quantities fed made no apparent difference in these few experiments. Therefore the growth curves have been arranged together. The maximum range and average body weight are only slightly below those for the males on the peanut meal diet, and appear to be within the limits of experimental error when so few experiments are used. The growth curve of the single female rat does not differ greatly from the rate of growth of the female rats on the peanut meal diet. It is 10 gm. below the lowest weight on the peanut diet. The bulkiness of the ether-extracted cottonseed kernel diet probably accounts for the very slightly smaller average daily intake of this diet (not shown on the charts), in which case, other things being equal, a slightly less rapid growth was to be expected. According to the literature, the utilization of cottonseed protein in animal feeding is not as complete as the utilization of protein in many other kinds of feed. However, it has been shown in the Protein Investigation Labora-

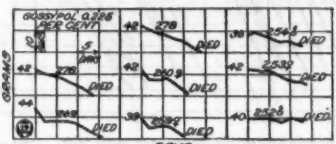

FIG. 3.—Effect on young rats of diet containing 0.225 per cent gossypol. Figure at left of curve indicates the weight at the beginning of the experiment; figure above the curve, the laboratory number of the animal. Because of short duration of experiments chart has been drawn on a scale twice that used in preceding and succeeding charts

tory of the Bureau of Chemistry that isolated cottonseed protein is as well digested *in vitro* as casein and arachin, the protein of the peanut (*5*).

The conclusion to be drawn from these experiments is that for the purpose of this study there was no significant difference in the rate of growth of rats fed the peanut meal diet and those fed the ether-extracted cottonseed kernel diet.

CONTROL DIET CONTAINING ADDED GOSSYPOL

That the diet was totally adequate has been shown. The growth curves in figure 3 show that young rats used in these tests failed to grow and eventually died on a diet containing 0.225 per cent of gossypol. On a diet containing 0.18 per cent two rats died rather soon, while two lived for some time (fig. 4). Recovery to some extent was begun when the diet was changed to the control food. From these few tests it is impossible to say with certainty that the subsequent decline on the control diet was connected with the previous gossypol feeding. From other experiments, however, it would seem that after the removal of gossypol from the diet, animals do not always recover their full health or normal growth rate. It is not certain in any of these tests that some of the results may not have been due to loss of appetite.

The rates of growth of rats upon diets containing 0.045, 0.0675, 0.09, and 0.135 per cent of gossypol are given in figures 5 to 9.

Only two experiments with a concentration of 0.045 per cent of gossypol were made. These were upon male rats. One rat appeared to have grown normally. The other was only slightly inferior to the rats in the control diet. On the eightieth day the average body weight of the two rats on the diet was only about 3 gm. below the average of the male rats fed ether-extracted cottonseed kernels The effect of this concentration of gossypol, therefore, if deleterious, is apparently too slight to be shown under the conditions of the experiments.

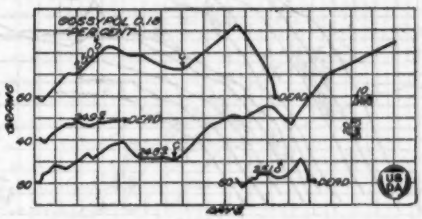

FIG. 4.—Effect of 0.18 per cent gossypol in the diet. Figures at the left of each curve indicate the weight at the beginning of the experiments, and that above the laboratory number of animal. "C" shows a change to control peanut meal diet

Upon a diet containing 0.0675 per cent of gossypol some retardation of rate of growth, as well as a slight tendency toward the attainment of a lower absolute degree of growth, was observed, as shown in the curves of body-weight averages. Some animals, however, made as good a showing as the poorest of the control rats. On the eightieth day the average weight of male rats was 238 gm. or 35 gm. less than that of the control rats on the peanut diet, and 21 gm. less than that of the rats on the diet containing ether-extracted cottonseed kernels. On the fortieth day the female rats averaged 134 gm., as compared with 137 gm. for those receiving the control diet. The growth curves of four rats (two males, No. 222 and 225, and two females, 223 and 224), the offspring of females reared on the

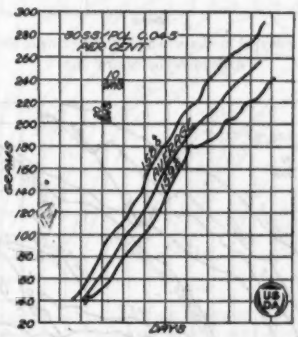

FIG. 5.—Effect of 0.045 per cent gossypol in the diet

diet containing 0.0675 per cent of gossypol and of the males reared upon the control diet, show that the rate of growth was below the average of that attained by the first generation fed upon the 0.0675 per cent gossypol diet, but was as good as that attained by the poorest on such a diet. It is evident, therefore, that the concentration of gossypol in the diet used in these experiments is close to the threshold value for toxicity under the conditions of the experiments. Whether or not it has any effect upon succeeding generations has not yet been determined.

A concentration of 0.09 per cent gossypol, as shown individually in four out of six experiments, produced a more marked retardation of growth. The result of

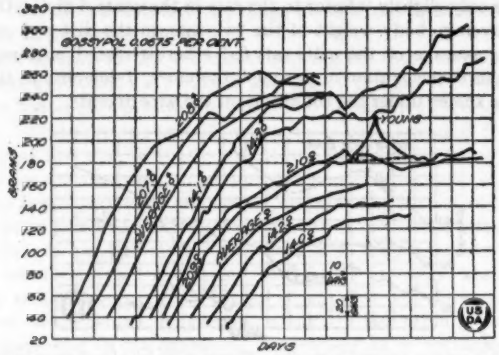

FIG. 6.—Effect of 0.0675 per cent gossypol in the diet. Broken line on one curve represents estimated weight had pregnancy not occurred

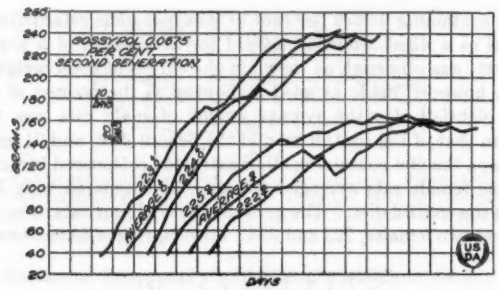

FIG. 7.—Effect of 0.0675 per cent gossypol in diet when fed to offspring of female rat raised on this diet and a male raised on control diet

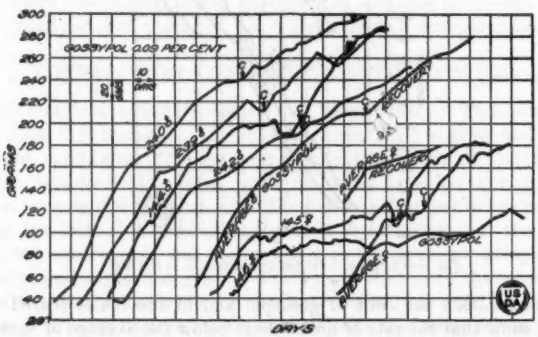

FIG. 8.—Effect of 0.09 per cent gossypol in the diet. "C" indicates change to control peanut meal diet. "Recovery" on "average" curves indicates period during which control diet was fed

the two remaining experiments is less marked. Two of the male rats when changed to the control peanut diet resumed growth immediately, although it was slower than in the case of the other two male rats. On the eightieth day the

four male rats averaged 207 gm., being 64 and 52 gm., respectively, below the average weight attained on the two control diets and 31 gm. below the average of the male rats fed on a diet containing 0.0675 per cent of gossypol. The average weight of the two female rats fed 0.09 per cent gossypol was below the average weight of the female rats on the other diets by 58, 38, and 35 gm., respectively.

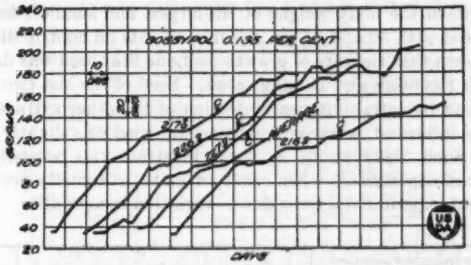

FIG. 9.—Effect of 0.135 per cent gossypol in the diet. "C" indicates change to control peanut meal diet

Only a few experiments were made with a concentration of 0.135 per cent of gossypol in the diet. The results, such as they are, indicate that the retardation of growth was marked and the resumption of growth poor. There is no question but that the poor resumption of growth is to be regarded as an effect of the gossypol, for the sturdiness of stock from which these rats came and the adequacy of the diet were certain.

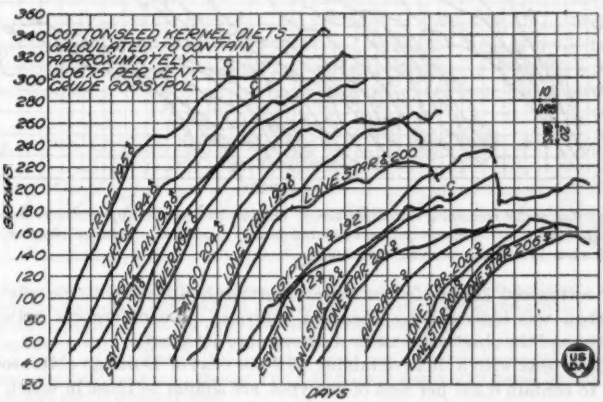

FIG. 10.—Effect of diet containing cottonseed kernels estimated to contain approximately 0.0675 per cent crude gossypol. "C" indicates change to control peanut meal diet

DIET CONTAINING ADDED RAW COTTONSEED KERNELS

The result of individual experiments in which raw cottonseed kernels were added to the diet in such proportions that the diet as a whole contained approximately 0.0675 and 0.135 per cent of gossypol are given in figures 10 and 11. Owing to the comparatively small number of experiments with each variety of kernel, separate average curves for each variety are not given. Instead, a composite curve for each sex for all kernels, based upon the average body weight of all the rats of each sex fed approximately the same estimated concentration of

gossypol, are presented. The rats fed cottonseed kernels grew on the average slightly better than the rats fed the estimated comparable quantities of gossypol.

In the case of the experiments with the cottonseed kernel diet estimated to contain 0.135 per cent of gossypol, the diet was withdrawn early and replaced by the control diet of peanut meal. The sudden change in the rate of growth is very marked, especially in the case of the males. On the sixtieth day the maximum difference between the body weight of the largest and smallest male rats was 70 gm. as compared with 30 and 35 gm. for normal rats on control diets.

It is also shown that the rate of growth on Lone Star seed was decidedly poorer than that with Egyptian and Durango seed. Seed of the last two varieties, however, were found by intraperitoneal injection of the ether extract to be as toxic as the analysis indicated, whereas the Lone Star seed was slightly less toxic than indicated. Such observations as these illustrate the necessity for a much larger number of experiments when exact estimates of toxicity are to be made in order to rule out experimental error due to variation in animals.

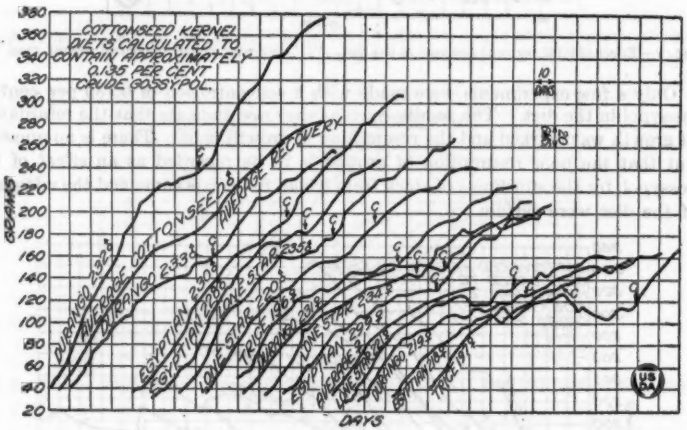

FIG 11.—Effect of diet containing cottonseed kernels estimated to contain approximately 0.135 per cent crude gossypol. "C" indicates change to control peanut meal diet. "Average recovery" indicates growth when all rats were receiving control diet

The cottonseed kernels fed in these experiments appeared to be slightly less toxic than was indicated by chemical analysis. The differences between the predicted and the observed toxicity, however, are not great.

Feeding tests with a diet containing 26.2 per cent of Durango cottonseed, estimated to contain 0.257 per cent of gossypol, are similar to those in which the diet contains 0.225 per cent of gossypol. Although in the Durango diet the estimated quantity of gossypol was a tenth greater than it was intended to be, this excess is within the limits of experimental error. The Durango seed, therefore, appears to be about as toxic as the gossypol analyses would indicate. The curves are given in figure 12.

The curves representing the rate of growth upon a diet containing 19.2 per cent of Egyptian cottonseed kernels, estimated to contain 0.227 per cent of gossypol, also presented in figure 12, are in practical agreement with the results of the tests in which the diet contained 0.225 per cent of added gossypol. The only difficulty in the way of stating that they are absolutely identical is the fact that one of the five rats on the Egyptian diet lived about twice as long as any of the

other rats. Since the significance of this single exception is not apparent, it may be concluded that for all practical purposes the Egyptian seed was as toxic as was indicated by the gossypol analysis.

The results of the feeding tests upon a diet containing 42.2 per cent of Lone Star cottonseed, estimated to contain 0.218 per cent of gossypol in the diet as a whole, are given in figure 12. They indicate that the actual toxicity is about equivalent to the toxicity of a diet containing 0.18 per cent of pure gossypol. By

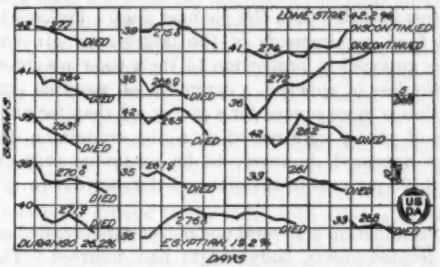

FIG. 12.—Effect of diet containing cottonseed kernels and estimated to contain approximately 0.225 per cent crude gossypol. Numbers to left of curve indicate the initial weight in grams and numbers above or below curve the laboratory number of animal. Because of short duration of experiments chart has been drawn on a scale twice that used in preceding chart

these tests, therefore, the Lone Star seed seems to be about 20 per cent less toxic than would be estimated from the gossypol analysis.

No experiments were made with a diet containing 54.6 per cent of Trice cottonseed kernels (estimated to contain 0.224 per cent of gossypol). In their place, experiments with 15 and 30 per cent of Trice kernels, the diets estimated to contain, respectively, 0.062 and 0.125 per cent of crude gossypol, are reported (fig. 13). These experiments, however, must be considered apart from those already re-

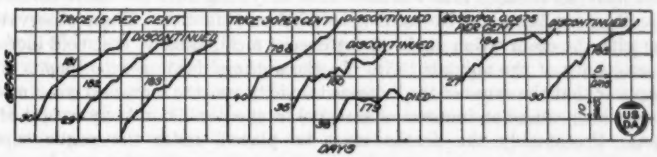

FIG. 13.—Effect of diet containing Trice cottonseed kernels estimated to contain approximately 0.0675 and 0.135 per cent crude gossypol, compared with a diet actually containing 0.0675 per cent gossypol. The eight experiments represented in this chart were conducted on rats from a single litter. They were performed during an interval when the laboratory was rather cool at night, and are therefore to be considered apart from others herein reported, in which the same diets were fed during warm weather or when the laboratory temperature regulator was in use. Because of the short duration of these experiments, the chart has been drawn on scale used in preceding chart

ported in which the same quantities of Trice cottonseed kernels were used during the warm weather. These experiments were performed on a single litter of rats at a time when the laboratory was cool at night, a condition which lowered the resistance of the animals. Compared with rats of the same litter which were fed a diet containing 0.0625 per cent of gossypol at the same time, the conclusion is that the Trice seed was probably from 67 to 75 per cent as toxic as was estimated from the gossypol analysis.

REPRODUCTION, STUNTING, AND SENSITIVITY DUE TO AGE, TEMPERATURE, AND APPETITE

Grown rats, some of which had been raised on control diets, as well as some which had been raised on diets containing a small quantity of cottonseed kernels or gossypol, were fed the more toxic concentrations of cottonseed kernels or gossypol. The results of these tests vary somewhat and are not sufficient in number to warrant detailed consideration. Nevertheless, they show that the older rats were less sensitive than the younger ones. Perhaps this is due to the relatively small food requirements of the larger rats. In consequence, they would ingest less gossypol in proportion to their body weight than the smaller rats. Perhaps the loss of appetite, a symptom of gossypol intoxication, was a factor, for inanition is more serious for a young rat than for an old one.

Several litters of young were reared on a diet containing 0.0675 per cent of gossypol. There appeared, however, to be an undue proportion of breeding failures. With 0.135 per cent in the diet no litters were born, although breeding was attempted.

Post-mortem examination of several undersized rats revealed nothing abnormal except when a decided loss in body weight had occurred. The tail and body lengths and the weight of undersized rats which had not lost body fat agreed with those reported by Donaldson (*4*). The weights of the heart and liver were also within the normal limits for rats of their size. This touches upon a practical problem in nutrition as yet unsolved—the criterion by which stunting of a mild type and without evidence of malnutrition or disease is to be determined. Thus a possibility to be considered is that the feeding of certain quantities of cottonseed products containing gossypol under some conditions, while apparently compatible with perfect health, may produce undersized animals.

Temperature affects the resistance of animals to gossypol. In the winter and cool spring weather when the laboratory was not well heated at night young rats and grown mice succumbed to concentrations of gossypol which otherwise would have survived. This was also true of young rats fed diets containing raw cottonseed kernels. No mice were tried on this diet. These observations agree with the observation made on cottonseed meal, namely, that cold increases the sensitivity of the animals to it. In the authors' feeding experiments it did not appear that the cause of the increased toxicity was the increased consumption of food which caused an increase of gossypol intake per kilo of body weight of the animal. Presumably the deaths must be placed under the vague caption "exposure."

Gossypol and raw cottonseed kernels markedly affect the appetite, although the effect is not regular. It appears that one gets the normal weight variation plus a variation due to the effect of gossypol on the appetite. This is shown in some groups of animals in which the difference between the better and the poorer growths is greater in the case of animals fed gossypol or cottonseed than in those on the control diet.

The extent of growth followed the appetite or food intake. Comparisons, however, were made only when approximately the same concentrations of gossypol or comparable quantities of cottonseed kernels are involved and for the same sex. The food intake diagrams are not presented.

DISCUSSION

The experiments in which pure gossypol dissolved in peanut oil was injected intraperitoneally and those in which the ether extract of different varieties of cottonseed was injected intraperitoneally show a general similarity in toxicity. The Egyptian and Durango seeds showed the toxicity that was to be expected from their gossypol content. The Lone Star and the Trice seeds, however, deviated somewhat from the expected toxicity. Chemical analysis of the seeds studied showed an extreme range in gossypol content of 300 per cent, whereas the range in toxicity of the ether extract from these seeds was from 400 to 450 per cent. This was due, not to a toxicity of the seeds in gossypol greater than that corresponding to their gossypol content, but rather to a lower toxicity of the seed relatively low in gossypol. It is to be expected, of course, that where the gossypol content is low, the errors of the analytical method will be exaggerated as compared with those seeds in which the gossypol content is high. Furthermore, it is to be expected that decomposition and modification of the gossypol will be relatively greater in the seeds with a low gossypol content than with those having a high gossypol content. These are the main factors in accounting for the differences between the calculated and the observed toxicity of different quantities of seed. The conclusion that the experiments with the intraperitoneal injection of gossypol itself and of ether extract of cottonseed show that the two are equally toxic and that gossypol is the only or at any rate the predominant toxic principle of cottonseed, seems to be warranted.

The feeding experiments with diets containing either added pure gossypol or raw cottonseed kernels added to the diet in such proportion that the gossypol content of the total diet was known show the same parallelism between gossypol content and toxicity. For diets containing quantities of gossypol large enough to cause death or arrest growth in a relatively short time this is entirely clear. With smaller quantities in the diet such parallelism, of course, is much less clear and is not to be expected. The symptoms observed from feeding gossypol and cottonseed kernels were similar. They consisted of loss of appetite and depression of growth, which, if sufficient quantities of gossypol or of cottonseed kernel had been fed, often led to death, if the feeding was continued. If the feeding was discontinued and the animals changed to control diet, growth was resumed at varying rates. There was some evidence that animals were permanently injured when fed for long periods on the gossypol or raw cottonseed kernel diets.

The results herein recorded could not have been due to any defect of the diet as such. A superabundance of protein, of vitamin, of salt, etc., was furnished so as to give the best opportunity for growth and to diminish as much as possible any indirect effect of the poison. Moreover, when additions were made to the control diet, care was taken to keep the protein content of the diet practically the same. It is obvious that merely to have added cottonseed products to the control diet would have changed its character, particularly with respect to protein, and would have introduced a further variable into the experiment.

Perhaps the results obtained in this investigation explain the discrepancies between the experiments of Richardson and Green (*8*) and those of Osborne and Mendel (*6*) with 9 per cent of protein (in the form of cottonseed flour) in the diet. Different degrees of growth were obtained. The reason for this may lie in the gossypol content of the different samples of cottonseed flour used by these investigators. At that time it was impossible to estimate the gossypol content, for no methods had been developed.

It is evident that henceforth a knowledge of the gossypol content is essential in any investigation of the toxicity and nutritional value of cottonseed press cake. Moreover, the data presented in this paper do not permit generalizations concern-

ing the toxicity of cottonseed press cake. Its toxicity will vary greatly with the variety of seed and with the method of preparation and subsequent treatment of the press cake. Moreover, the rat is a relatively resistant animal. In the experiments herein reported, as well as in some of the experiments reported in the literature, conditions very favorable to the well-being of the animal were usually provided. Before generalizations concerning the toxicity of cottonseed products can be made, further work must be done upon other species of animals and upon animals kept under different conditions.

SUMMARY

A comparison of the toxicity of pure gossypol with that of four varieties of cottonseed kernels was made. The cottonseed kernels contained known quantities of gossypol. Pure gossypol in peanut oil solution and the ether extract of cottonseed kernels were injected intraperitoneally into rats. The toxicity of the cottonseed kernel extracts corresponded to their gossypol content with moderate variations. Rats were fed upon a totally adequate diet to which were added in some cases known quantities of gossypol and in other cases raw cottonseed kernels in which the gossypol content had been determined. The toxicity of these diets with moderate variations corresponded to their gossypol content.

The results herein reported bear out the conclusion that the toxicity of cottonseed kernels is due to their gossypol content. Inasmuch as the gossypol content of cottonseed kernels varies in the different producing regions, the observed regional variation in the toxicity of raw cottonseed kernels is explained.

Accordingly, this tendency to geographic variation in the toxicity of the raw material would be expected to affect the toxicity of cottonseed press cake and meal made in different regions by the same manufacturing process. Confirmation of this conclusion is found in the fact that in the Atlantic Coast States, where the gossypol content of cottonseed averages slightly less than 1 per cent, cottonseed poisoning is more feared than in the Southwest, where gossypol occurs in cottonseed in smaller quantities.

In this investigation no attempt has been made to study the variations in toxicity which might be due to different methods of treating the seed in the manufacturing process. No attempt was made to study the special procedures of individual investigators in this field. The effort has been limited to establishing the toxicity of the raw material used in the manufacture of the meal. Differences in this respect are at present beyond control. They must be taken into account both in research upon this subject and in the practical use of the material in industry and agriculture. Finally, an experimental basis has been found which makes it possible to correlate the divergent views and the apparently contradictory findings of various investigators.

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PHARMACOLOGY OF GOSSYPOL[1]

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INTRODUCTION

The gossypol content of cottonseed kernels varies in the different cotton-producing regions of the United States (*9*)[2] and the toxicity of different lots of kernels is directly proportional to their gossypol content (*10*). The rats used as test animals for the experiments on which these conclusions were based, however, did not exhibit all the manifestations of the cottonseed poisoning of farm animals, namely, diarrhea, loss of appetite, loss of weight, shortness of breath, and paralysis. Edema of the anogenital region also sometimes occurs and post-mortem examination may reveal effusion into the serous cavities, edema of the lungs, hypertrophy of the heart, and neuritis. Rommel and Vedder (*8*) have pointed out the resemblance of this symptom complex to beriberi. Of all these effects of cottonseed poisoning rats showed only loss of appetite and loss of weight. To remove any doubt as to the identity of the agent causing these intoxications of rats and of the causative agent of cottonseed poisoning of farm animals, experiments upon animals other than rats were undertaken.

ANIMALS USED

As the Bureau of Chemistry has no facilities for experiments on farm animals, the work was done on cats which are carnivorous and are particularly well suited to experiments in which inanition may become a complicating factor because they are less likely to succumb to acidosis than herbivorous or omnivorous animals. The results of this investigation upon the toxicity of gossypol for cats, together with some additional observations on rabbits, guinea pigs, rats, and mice, are here presented.

RATIONS FED

In the rabbit feeding experiments the gossypol was added in ether solution to the oats of the diet, the ether being removed subsequently by evaporation. A small ration of carrots or cabbage was also fed daily. Since this treatment of oat kernels presumably causes the gossypol to be deposited on the oat hulls, rabbits which hull their oats were rejected. In the cat feeding experiments, meat to which gossypol dissolved in butterfat had been added was refused. The solution of gossypol in butterfat was therefore mixed with skim milk powder and this mixture was incorporated in the hashed lean meat upon which the cats were fed. Meat is moist and does not necessarily take up gossypol as would dry material and it is impossible to work up the hashed meat and the milk powder mixture into a homogeneous mass. This method of administering gossypol therefore is not ideal when the effect of gossypol upon digestion is being studied. In all of the feeding experiments, except those performed to determine the effect of different concentrations, the dosage was so adjusted as to feed the maximum quantity of gossypol compatible with the maintenance of a fair appetite.

[1] Received for publication Mar. 21, 1924.

[2] Reference is made by number (italic) to "Literature cited," p. 197.

EFFECT OF GOSSYPOL

One of the effects of feeding gossypol was diarrhea. Another was loss of appetite, which usually occurred soon after the administration of as little as 450 parts per million in the diet to both cats and rabbits, although some rabbits withstood this concentration for a time. The highest concentration on which the appetite of rabbits was maintained was 225 parts per million of gossypol in the diet. No disturbances of appetite in cats were noted from 112 parts of gossypol per million of diet, but with 225 parts loss of appetite was common. In comparing the two species allowance must be made for the high moisture content of the cat diet as well as for the absolute bulk of food eaten. Rats apparently are decidedly less sensitive, for they withstand from three to six times greater concentrations of gossypol in the diet, despite the fact that in proportion to their size they consume larger quantities of food than cats or rabbits (*10*).

METABOLISM OF ANIMALS UNDERGOING GOSSYPOL POISONING

A study with gossypol similar to that of Wells and Ewing (*11*) on the metabolism of animals undergoing cottonseed poisoning seemed desirable. Especially did it seem important to maintain the appetite of the test animals in order to avoid the nitrogen fluctuation which in the experiments of Wells and Ewing may have obscured one of the effects of the intoxication. While some trouble was encountered in keeping the animals eating the entire ration, this was done successfully in the case of one cat, although after several feedings of gossypol, the ration was not consumed as rapidly as before. There was also some trouble from diarrhea. This was controlled by feeding bone ash and also by cutting the food allowance to the minimum.

The data on the intake and outgo of nitrogen are given in Table I. During the laboratory hours the urine was collected when voided, and preserved. At the end of the periods, which lasted from one to several days, the urine was always pressed out of the bladder and caught in a graduated cylinder. Only gentle house-broken female cats were used. The reliability of this procedure for dividing the experiment fairly accurately into periods of 24 hours or multiples thereof was also demonstrated by data on control periods. Many times no urine was voided during the 24 hours. Complete specimens were therefore pressed out into the cylinder at one time. A charcoal marker was used so that the feces could be separated. Usually periods longer than those used in collecting the urine were employed.

It seems justifiable to conclude that for the period during which the diet consisted of a daily ration of ground lean meat, prepared at the beginning of the experiment, the administration of gossypol caused a negative nitrogen balance, the animal in the fore-period having been practically in nitrogen equilibrium. This conclusion is in harmony with the results of Macy and Mendel (*5*). The animals to which they fed cottonseed or cottonseed meal generally lost more weight than the control animals which in each of their experiments received exactly the same quantity of food as that eaten the previous day by the test animals.

The interpretation of the data on the fecal nitrogen is less clear, owing to a lack of certainty of precise separation of feces by markers, and the slightness of the increase of fecal nitrogen when gossypol was fed. Moreover, the markers may have had some effect on the fecal nitrogen. It seems probable, however, that the administration of gossypol caused a somewhat increased elimination of nitrogen through the feces. It is less than is to be expected from the results of Jones and Waterman (*4*), Crowther and Woodman (*3*), Mendel and Fine (*6*), and Rather (*7*). Jones and Waterman, using gossypol prepared by the writers, found that it retarded the digestion of cottonseed globulin and of casein *in vitro*.

TABLE I.—*Metabolism of cat No. 30 undergoing gossypol poisoning*

DIET: 100 GM. MEAT, 2 GM. BUTTERFAT, 2 GM. SKIM MILK POWDER

Date	Weight	Bone ash fed	Nitrogen daily				Gossypol administered
			Fed	Urine [a]	Feces	Balance	
1920	*Grams*	*Grams*	*Grams*	*Grams*	*Gram*	*Gram*	
Oct. 26–31	1,820 / 1,855	2	3.42	----------	0.077	----------	Fore-period.
Nov. 1–4	1,890 / 1,985	2	3.42	----------	.312	----------	90 mgm. daily.
5	1,890	2	[b] 2.01	----------	.055	----------	
6–7 [c]	1,795 / 1,825	4	3.33	----------	.192	----------	
8	1,825	4	3.33	2.641	.192	+0.497	None.
9	1,830	4	3.33	2.697	.143	+.490	
10	1,820	4	3.33	2.725	.143	+.462	
11 [c]	1,825	4	3.33	2.599	.206	+.525	
12	1,880	4	3.33	2.599	.206	+.525	
13 [c]	1,865	4	3.33	3.117	.206	+.007	
14 [c]	----------	4	3.33	3.117	.206	+.007	45 mgm. daily.
15	1,855	4	3.33	3.302	Lost.	----------	
16	1,865	4	3.33	3.302	...do...	----------	
17	1,850	4	3.33	3.397	...do...	----------	
18	1,860	4	3.33	3.397	...do...	----------	

DIET: 75 GM. MEAT, 2 GM. BUTTERFAT, 2 GM. SKIM MILK POWDER

Dec. 11	1,843	2	2.617	2.640	0.061	−0.084	
12	----------	2	2.617	2.640	.061	−.084	
13	1,835	2	2.617	2.640	.061	−.084	Fore-period.
14	----------	2	2.617	2.515	.061	+.041	
15	1,840	2	2.617	2.515	.061	+.041	
16	1,840	2	2.617	2.690	.182	−.255	
17 [c]	1,875	2	2.617	2.599	.182	−.164	
18	----------	2	2.617	2.802	.182	−.367	45 mgm. in butterfat and milk powder daily.
19	----------	2	2.617	2.802	.182	−.490	
20 [c]	1,840	2	2.617	3.068	.245	−.696	
21	1,850	2	2.617	3.068	.245	−.696	
22	1,820	2	2.617	3.068	.245	−.696	
23 [c]	1,780	2	2.617	3.068	.245	−.696	
24	1,780	2	2.617	2.456	.1414	+.020	After-period.
1921 Jan. 4	1,765						

[a] No albuminuria.
[b] Net quantity fed after animal vomited.
[c] 1 gm. of charcoal fed.

TABLE II.—*Toxicity of gossypol*

Animals used	Injected intraperitoneally in peanut oil			Animals used	Injected intravenously as sodium salt		
	Usually survived	Average lethal	Certain lethal		Usually survived	Average lethal	Certain lethal
	Mgm. per kgm.	*Mgm. per kgm.*	*Mgm. per kgm.*		*Mgm. per kgm.*	*Mgm. per kgm.*	*Mgm. per kgm.*
5 rabbits	5	10	20	10 rabbits	----------	35–40	50–
2 cats	40–80	----------	----------	9 cats	50	----------	75
20 rats	20	30–35	50	8 rats	35	40–50	50+

The other investigators reported a low digestibility of the protein in cottonseed as measured by fecal nitrogen. The smallness of the increase found in the present investigation may be due to the manner of administering the gossypol. Perhaps it did not come in close enough contact with the meat protein of the cat's food. Moreover, the experiments herein reported were conducted so that absorption was favored by cutting the diet down to a low level and by checking the tendency to diarrhea with bone ash. In an experiment conducted without bone ash and with a large dose of gossypol the diarrhea was marked and the fecal nitrogen was high.

PARALYSIS FROM GOSSYPOL

When the gossypol content of the rabbits' diet was adjusted to between 225 and 450 parts per million, two of the rabbits which retained a fairly good appetite developed spastic paralysis of the hind legs within two months. The hind legs were stiff and protruded forward upon the abdomen (similar to cat shown in Pl. 2, B). The anogenital region became edematous and ulcerated and there was dribbling of urine. Paralysis also occurs in cats (Pl. 1 and 2), the hind legs being more often affected. In several cases in which only the fore legs seemed to be affected there was reason to suspect that the hind legs were also involved, although not enough to be apparent. The paralytic condition may become progressively worse for a time after the gossypol diet is discontinued or may improve while the gossypol diet is being continued. The kangaroo hop (Pl. 2, C and D) illustrates the effort of the animal to compensate for the paretic condition. Even the tail is brought into play as an accessory organ of locomotion.

Paralysis was observed not only in rabbits continuously fed gossypol but also in one rabbit which survived intraperitoneal injection of 5 mgm. of gossypol per kilo dissolved in peanut oil, and in one receiving orally 50 mgm. of gossypol per kilo dissolved in peanut oil. Only these two individuals out of a large number of rabbits which were injected showed paralysis. The paralysis developed within 10 to 20 days, being preceded only by weakness. Within several more days the hind legs became spastic and extended. The hind legs were affected unequally. The inequality was especially well shown in the behavior of the tail, which turned to one side. Almost complete recovery occurred within two months.

Although on injecting other animals the paralysis could not be reproduced, it is believed that the paralysis of the two rabbits described was due to gossypol, for among the several thousand rabbits observed in the pharmacological laboratory of this Bureau spontaneous paralysis has never been encountered. Moreover, the paralysis resembled exactly that found on feeding gossypol. It is believed that these observations on the feeding or injection of gossypol are the first recorded instances of a paralysis in rabbits which is attributable to gossypol or to cottonseed products.

Gossypol has a paralytic effect not merely upon the neuromuscular apparatus of striated muscle but also upon that of smooth muscle. At any rate it produces definite paralysis of the isolated rabbit intestines in concentration of about 1 to 5,000 within about 15 minutes. With more dilute solutions, such as 1 to 100,000, he effect was not so certain. Post-mortem examination of rats dead of intraperitoneal injection of gossypol frequently reveals a condition resembling paralytic ileus. As cottonroot bark which contains gossypol is regarded as an abortifacient, some effect upon the smooth muscle of the uterus might be anticipated. However, while no experiments were made upon the isolated uterus, abortion could not be effected in pregnant cats or rabbits by poisoning them with gossypol. The few fetuses which were born succumbed and presumably would have been born anyway. In one case only part of the litter was born. When pregnant animals poisoned by gossypol were sacrificed, live fetuses were found and no separation of placentae was observed.

In the continued feeding experiments with rabbits evidence of the action of gossypol on the neuromuscular apparatus is found in the dribbling of the urine. This, however, was associated with motor paralysis, so that the point of attack might have been in the cord.

EFFECT OF GOSSYPOL ON CIRCULATION

Gossypol apparently also affects the neuromuscular apparatus of the heart, for frogs that have died from the slow absorption of gossypol injected in oil solution into the ventral lymph sac show a peculiar spotted condition of the ventricle which presumably is associated with the fact that portions are in systole or rigor while other portions are fully stretched. In higher animals death comes from cardiac failure. Primarily the respiration is not at all or only slightly affected.

Gossypol is a circulatory depressant. It produces a fall in blood pressure, usually immediately after it is injected. A recovery of pressure may occur gradually. Section of the vagi may bring it back to normal immediately, or, especially in rabbits, to above normal. For some time after injections of gossypol the heart shows skipped or weak beats. Once a two to one block was obtained which later disappeared spontaneously. Nevertheless, two cats receiving gossypol in continuous feeding experiments which died under observation gave no clinical evidence of irregularity of the heart rhythm. One cat, a good feeder, which received gossypol continuously and which died with edema of the lung, had decided shortness of breath and rapid pulse and breathing before death. The enlarged heart is shown in Plate 2, a. Several other cats showed the same symptoms.

EDEMA FROM GOSSYPOL

Edema may follow gossypol injections as well as gossypol feeding. Marked edema of the ears of rabbits develops when gossypol solutions are injected into the ear veins, presumably owing to a slight leakage of the solution into the perivascular tissue. Edema also occurs around oil solutions of the gossypol in the subcutaneous tissue. Marked serous exudation follows in cats, rabbits, and rats when peanut oil solutions are injected intraperitoneally. In several cats which survived and subsequently were examined the ascites was intense and protracted. The injection of the oil free from gossypol does not ordinarily give rise to any visible serous exudate in the case of rats and only very little in the case of cats or rabbits.

When gossypol is injected intravenously in alkaline solution, or intraperitoneally in oil solution, edema of the lungs occurs in rabbits, guinea pigs, cats, rats, and mice. This is more intense and appears sooner the larger the dose. The fatal edema is hemorrhagic and red exudation may appear at the nose. Edema of the lungs also occurs in cats in chronic intoxication from gossypol feeding. Some effusion into the serous cavities may be noted. Measurements were made of the limbs of paralyzed cats but no disproportion was evident. Upon recovery from paralysis there was possibly a slight decrease in the size of the limbs, perhaps connected with the disappearance of an edema.

POST-MORTEM EXAMINATION

Data on the toxicity of gossypol administered intravenously in fixed oil are given in Table II. The rabbits which died did so usually within one to two days, whereas the cats, except those given large doses, lingered for four to five days. The time of death of the rats varied.

Examination by the Marchi method of the sciatic nerve from several paralyzed cats revealed degeneration. Dr. M. C. Winternitz, who also examined the experimental animals of Macy and Mendel (*5*), very kindly made histological

examinations of the lungs of four rabbits dying from the intravenous injection of 50 mgm. of gossypol per kilo in filtered alkaline solution. He reported in part as follows:

> I find in three of the animals (rabbits Nos. 114, 118, and 119) a congestion of the alveolar capillaries, fairly diffuse edema of the lung, and in several sections extravasations of red blood cells into the albuminous exudate within the alveoli. On the other hand, rabbit No. 117 shows a relatively normal lung tissue. In one animal (rabbit No. 114) polymorphonuclear leukocytes and fibrin also occur in small foci. The pneumonic areas in this rabbit are the more interesting because there is no exudate in the lumen of the bronchi. In rabbit No. 118 there is a relatively slight admixture of either red or white cells and fibrin in the exudate. In rabbit No. 119 there is no outspoken purulent pneumonic condition.

The frequent lesions observed in Macy and Mendel's series of subacute and chronic intoxication were congestion and hemorrhage in the intestines, congestion of the liver and kidneys, and edema of the lungs. The lungs examined by Doctor Winternitz for the writers came from animals that had succumbed to acute intoxication. One animal died before pulmonary complications had time to develop. Three animals showed fairly diffuse edema, congestion of alveolar capillaries, and, in a few places, extravasation into the exudate in the lumen of the alveoli. The pneumonic condition was secondary. Marked cardiac enlargement (Pl. 2, a) was observed in the cat described as having had dyspnoea before death. The other cats with similar symptoms did not give the positive evidence of cardiac enlargement that was shown by this animal.

SUMMARY

Gossypol has been fed for long periods to cats and rabbits in dosages small enough to avoid marked loss of appetite, yet large enough to produce chronic intoxication. The symptoms and lesions observed were loss of appetite with the larger dosages, paralysis, with nerve degeneration, shortness of breath, cardiac hypertrophy, edema of the anogenital region and of the lungs, and effusion into the serous cavities. Spontaneous recovery may occur temporarily while gossypol is being administered and the condition of the animal may become progressively worse for some time after it is discontinued.

Following the subcutaneous, intravenous, and intraperitoneal injection of gossypol, there is local edema of the lungs, which may be hemorrhagic, and effusion into the serous cavities. Blood pressure falls, heart action becomes irregular, and death comes from cardiac involvements.

Pure gossypol is capable of producing nearly all the manifestations reported as characteristic of the cottonseed intoxication of farm animals. Therefore the conclusion seems warranted that gossypol is the principal causative agent of cottonseed poisoning. This conclusion is supported by the fact, established in earlier papers of this series (*9*, *10*), that the toxicity of raw cottonseed kernels varies nearly directly with their gossypol content, or is only slightly less. Thus there can not be much of any other toxic substance in cottonseed kernels unless it be some decomposition product of gossypol. The pharmacology of such products has not been investigated.

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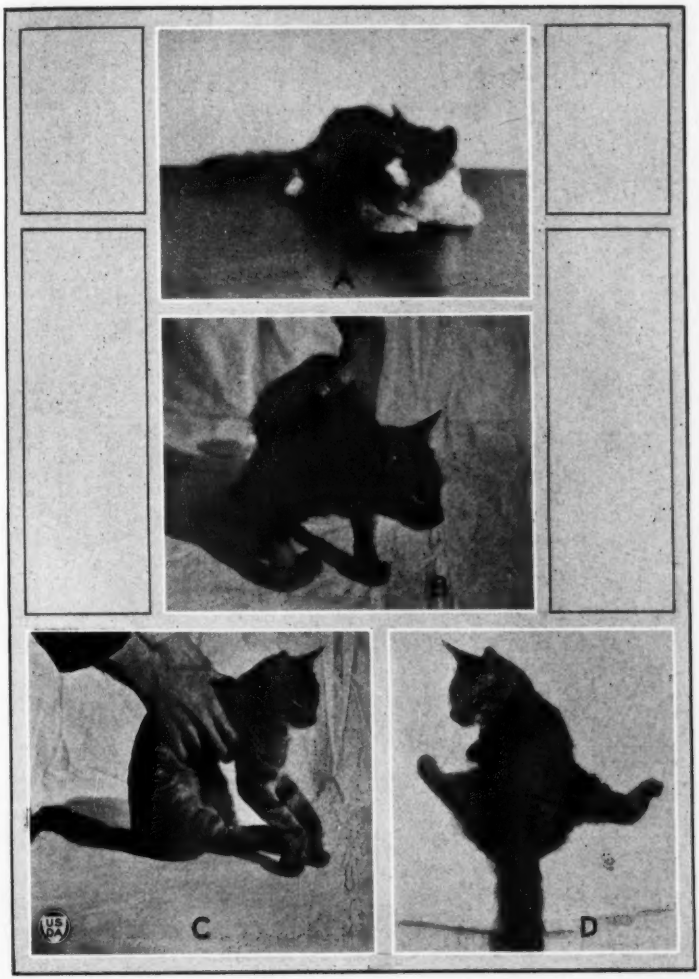
PLATE 1

A.—Early stage of paralysis of fore legs, with the inward curl. The legs become crossed when the animal is raised.

B.—Unequal paralysis of fore legs. This position was often assumed as the cat walked.

C.—Double-jointed effect often seen as the cat walked.

D.—Spastic paralysis of unequal degree in hind legs of cat No. 11 (September 22, 1920). The tail was completely extended. Locomotion was accomplished by the fore legs, the buttocks and abdomen being dragged on the floor. Rabbits also show this type of paralysis. In earlier stages, paralysis was of a more flaccid type.


A
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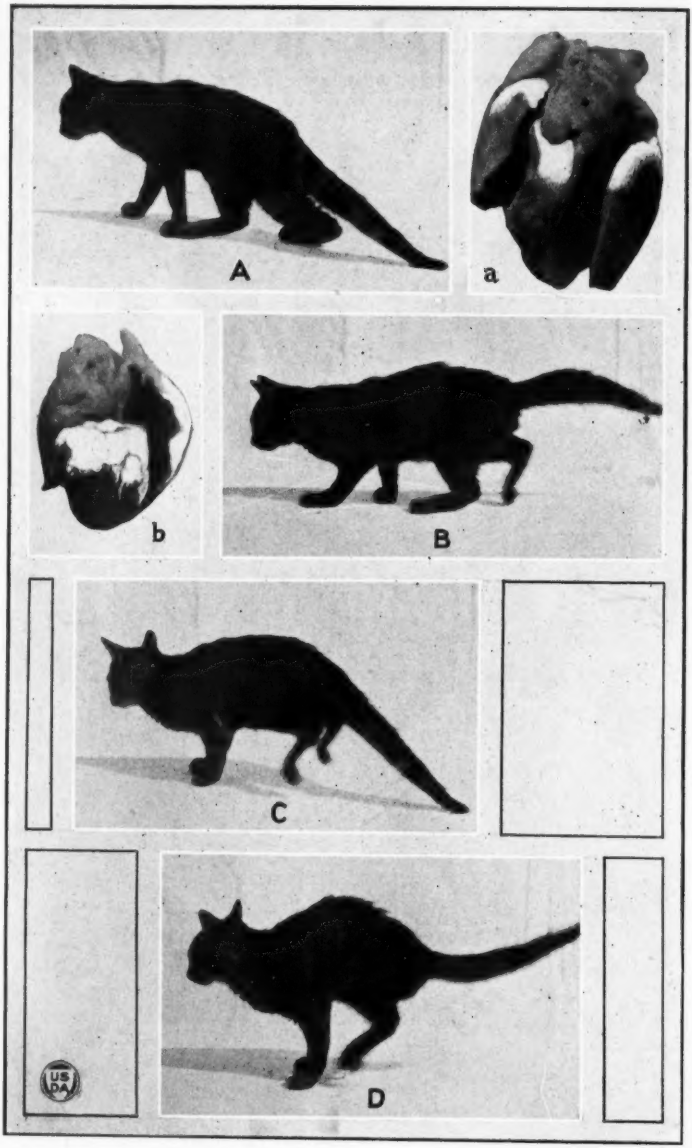
A
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PLATE 2

A and B.—Partial recovery of cat No. 11 (October 14, 1920) from stage shown in Plate 1. It has a slight weakness of the fore legs, a greater weakness of the hind legs, and the quick step of the multiple neuritis type of paralysis. (Negative exposed for one-fiftieth of a second.)

C and D.—Unusual form of rapid locomotion shown by cat No. 11 (October 14, 1920). The back is arched and the tail is used as an aid in locomotion. (Negative exposed for one-fiftieth of a second.)

a and b.—(*a*) Enlarged hypertrophied and dilated heart of cat No. 11, and (*b*) heart of cat No. 19 (control animal). The large heart (*a*) weighed 12.35 gm. The net body weight of cat No. 11, which was about 32 weeks old, was 1,475 gm., exclusive of the serous exudate (215 gm.). Its previous maximum body weight was 1,890 gm. The body weight of cat No. 19, about 29 weeks old, was 2,405 gm. and the weight of its heart was 6.1 gm. The weight of the enlarged heart was twice that of the heart of a normal cat weighing 1,465 gm. The enlarged heart has much less epicardial fat than the smaller one.